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FORMULATION, CHARACTERIZATION AND EVALUATION OF CASPOFUNGIN EMULGEL FOR ITS ANTI-FUNGAL ACTIVITY

***[1]Barkha Raghav, [2]Vikas Sharma and [3]Shamim Ahmad**

*[1]Research Scholar, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

[2]Assistant Professor, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

[3]Professor & Director, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

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Corresponding Author: Barkha Raghav

Address: Research Scholar, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

Email ID: raghav.barkha@gmail.com

ABSTRACT

A target-oriented drug delivery system is referred to as a novel drug delivery system since it reduces the dose and increases effectiveness without endangering other organs. The present study was based on the formulation, characterization and evaluation of caspofungin emulgel for its anti-fungal activity. Emulgels were prepared and evaluated for parameters i.e., physical examination, pH determination, swelling index, drug content estimation and in-vitro drug release. The antifungal activity was evaluated through well diffusion method against fungus strains i.e., *Aspergillus niger, Penicillium notatum*, *Candida albicans* and *Rhizopus species.* In results, the pH level was determined to maximize solubility and absorption. pH was estimated as 6.3±0.2 in F1. While pH for F2 and F3 was measured as 6.2±0.1 and 6.4±0.3, respectively. After 6 hours, the % drug release from formulations F1, F2, and F3 was 81.31±0.11, 82.35±0.64, and 83.20±0.33, respectively. In F3, antifungal activity was recorded as 5.39mm, 6.29mm, 7.14mm, and 6.23mm in *A. niger*, *P. notatum, C. albicans and Rhizopus species,* respectively. It can be said that Caspofungin has more anti-fungal potential on *C. albicans* as compared to *A. niger.* It might be due to destruction of cell wall and/ nucleic acid of fungal strains. In conclusion, there is no question that capsofungin emulgels

will be the most sought-after dosage form due to its superior therapeutic potential and less patient issues.

KEYWORDS: Caspofungin, emulgel, formulation, antifungal, pH.

INTRODUCTION

A target-oriented drug delivery system is referred to as a novel drug delivery system since it reduces the dose and increases effectiveness without endangering other organs. The way a medicine is taken can have a big impact on how effective it is (Santini et al. 2000). A few of the drug carriers that have been created over the years include micelles, lipoproteins, cell ghosts, cells, and microcapsules. The carriers may be targeted, engineered to degrade gradually, and react to stimuli. "Targeting" is the process of directing a drug-infused system to a certain location (Kopecek, 2003).

Emulgels are very much effective in delivery of topical dermatological agents but they are not suitable for hydrophobic or lipophilic drugs. Moreover, the topical drug delivery system has been a very impactful way of pouring the medicine at the desired site without producing serious systemic outcomes (Aher et al. 2013). They also facilitate the bioavailability of drugs at target sites by leaving the first pass metabolism or pre-systemic metabolism occurring in Liver (Amar et al. 2022). In topical formulations, the release rate of preparations depends upon the physical, chemical properties and nature of carrier molecules. Release rate and stability of emulgels are dependent as per the nature and concentrations of polymers taken as gels (Asthana et al. 2016).

Caspofungin

The first member of a novel class of antifungal drugs known as echinocandins is capsofungin (Cancidas®)1. Treatment of invasive candidiasis, including candidaemia, in both neutropenic and non-neutropenic patients; empirical management of suspected fungal infections in febrile, neutropenic patients (Denning, 2003).

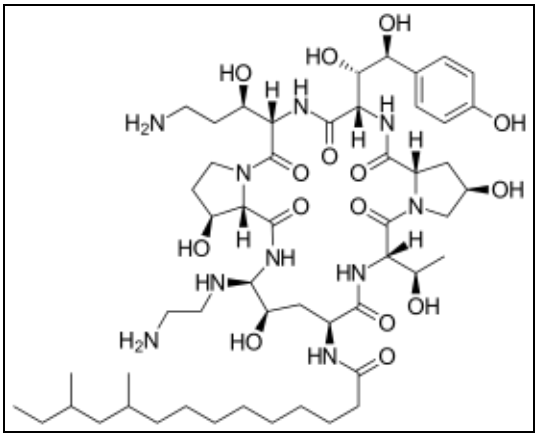


Fig. 1: Structure of Caspofungin.

Molecular Formula: $C_{52}H_{88}N_{10}O_{15}$

Molar mass: 1093.331 g·mol^{-1}

Caspofungin, also known as echinocandin, is a water-soluble, semisynthetic cyclic lipopeptide that prevents the synthesis of β-(1,3)-D-glucan, a crucial element of many fungi's cell walls but is absent from mammalian cells. A high-affinity transporter appears to be involved in C. albicans' uptake of caspofungin (Deresinski and Stevens, 2003). The fungal cell wall's β(1,3)-D-glucan synthase enzyme is inhibited by capsofungin in a non-competitive way, which prevents β(1,3)-D-glucan from being synthesized. One of the primary polysaccharides of the fungal cell wall is β(1,3)-D-glucan, which creates a solid three-dimensional matrix that affects the cell wall's shape and mechanical strength (Horan-Saullo and Alexander, 2016).

There are two benefits to inhibiting the formation of β(1,3)-D-glucan: fungistatic and fungicidal. The inhibition of cell wall formation results in a restriction of cell growth, which is known as the fungistatic effect. The fungal cell wall's altered integrity, the cell's loss of mechanical strength, and its inability to maintain internal osmotic pressure all contribute to the fungicidal effect (Latge, 2007).

MATERIALS AND METHODS

Experimental Requirements

Caspofungin (API), ethanol, carbopol 934, Tween 80, Span 80, Propylene glycol, clove oil, Methyl paraben.

Digital balance, magnetic stirrer, Spectrophotometer, compound microscope, Dissolution test apparatus, pH meter.

Formulation of Emulgel

a. Preparation of emulsion

Preparation of aqueous phase

The aqueous phase of the emulsion was prepared by dissolving Tween 80 in contaminated free water.

Preparation of Oil Phase

While capsofungin was initially dissolved in ethanol, methyl and propyl paraben were dissolved in propylene glycol. Aqueous solution is supplemented with these two solutions. The aqueous and oily phases were each heated to 75°C in isolation. After that, the aqueous phase and oil phase were combined while being constantly stirred until the mixture reached room temperature.

b. Preparation of gel

The gel bases are made by individually adding varying polymer concentrations to distilled water and continuously shaking the mixture with a mechanical shaker. Triethanolamine was used to raise each formulation's pH to 6-6.5.

c. Formulation of Emulgel

The prepared emulsion is mixed with the gel with gentle stirring to obtain the respective emulgel.

Evaluation parameters of Emulgel (Pani et al. 2014; Yadav et al. 2016)

Physical Examination

The prepared emulgel preparations were examined for their color, homogeneity, consistency, and phase separation.

Measurement of pH

Using a digital pH meter, the pH of the emulgel formulations was examined. One gram of emulgel was dissolved in one hundred milliliters of distilled water to create an emulgel solution, which was then set aside for two hours. Each formulation's pH was measured three times, and the average results were recorded.

Swelling Index

In this procedure, 1 gm of emulgel is taken on aluminum foil (porous) and then placed in a beaker containing 10 ml 0.1N Sodium hydroxide (NaOH). Then samples were removed out

from the beakers (at different time breaks) and put it on dry place. After samples were weighed again as they were dried.

Swelling index is calculated by using following formula-

$$\text{Swelling index (SW) \%} = [(Wt - Wo)/Wo] \times 100$$

Where,

Swelling index (SW) %= Equilibrium percent swelling

Wo= Original weight of emulgel, Wt= Weight of swollen emulgel

Drug Content Estimation

1 gm of emulgel is dissolved in 50 ml of 0.1N NaOH and then kept aside for 2 hr (reaction time). Then 5 ml of sample is withdrawn and absorbance measured at 276 nm wavelength by UV visible spectrophotometer.

***In-vitro* Drug Release**

The Franz diffusion (FD) cell uses phosphate buffer at pH scale to carry out in-vitro drug release. For diffusion, cellophane membrane is utilized as a semi-permeable membrane. 20 ml of medium are poured into the receptor compartment up until the collection limb mark. Following that, the membrane is retained on the receptor compartment.

1g of emulgel should be precisely weighed, placed between the donor and receptor compartments on the membrane, and fitted tightly. The donor compartment magnetic stirrer and the external stirrer's rotations per minute (rpm) are regulated to create laminar flow in the medium. The circulating water jacket keeps the FD cell's temperature at 37°C. 5 ml of sample is drawn from the collection limb at periodic intervals, and the same volume is then replaced with buffer media. The samples are then examined by a UV spectrophotometer at a wavelength of 276 nm to determine concentrations.

Evaluation of anti-fungal activity

By using the well diffusion method, the antifungal activity of emulgel against fungus was identified. Nutrient Agar broth was utilized to cultivate a 24-hour-old culture of fungus, which was then used to make a suspension of fungi. After sterilization at 121°C (1.05 kg/cm2 pressure) for 20 minutes, nutrient agar solution was added. A sterile spreader was used to cover the whole surface of the agar plates when we inoculated them with each fungal

suspension. With a sterile cork borer, 5mm wells were made in the solidified media, and each well was filled with emulgel. The diameter (mm) of the inhibitory zone surrounding the well was measured after 24 hours of incubation at 30°C. As a standard antifungal formulation, itraconazole gel (Itromed 1%) is utilized. Every antifungal study was carried out in triplicate. Fungus strains i.e., *Aspergillus niger*, *Penicillium notatum*, *Candida albicans and Rhizopus species* will be used for screening of anti-fungal activity (Kaushik et al. 2020).

RESULTS AND DISCUSSION

Pre-formulation studies

- **Solubility**

Caspofungin was evaluated for solubility in the various solvents. Methanol and ethanol showed freely solubility of Caspofungin. It was shown to be freely soluble in phosphate buffer, chloroform, and Tween 80.

Table 1: Solubility of Caspofungin.

Solvent	Caspofungin
Methanol	Soluble
Tween 80	Freely Soluble
Ethanol	Soluble
Distilled water	Freely soluble
Chloroform	Soluble
Phosphate Buffer	Freely Soluble

- **Extract excipients compatibility**

Drug-excipient compatibility spectral analysis was also conducted on Caspofungin, utilizing FT-IR spectroscopy both singly and in excipients.

The following is a log and demonstration of this compatibility:

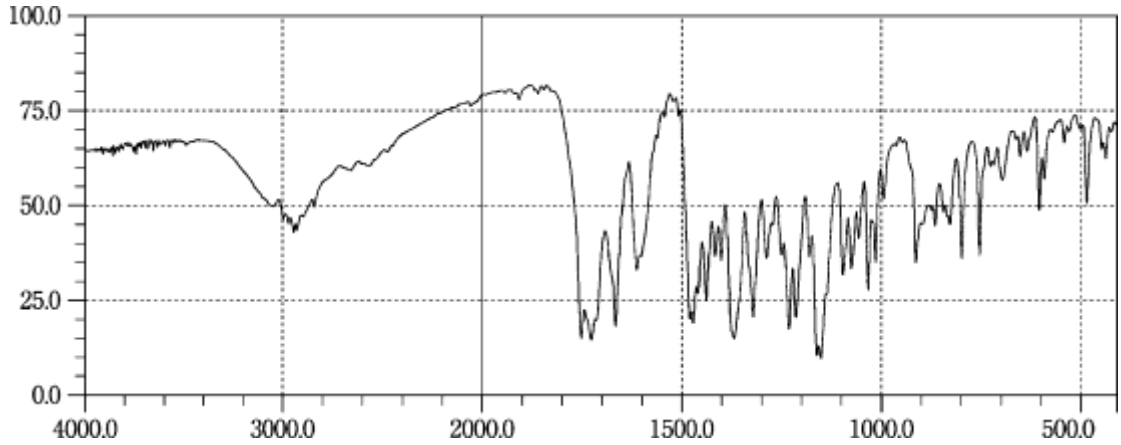


Fig. 2: FTIR of Caspofungin.

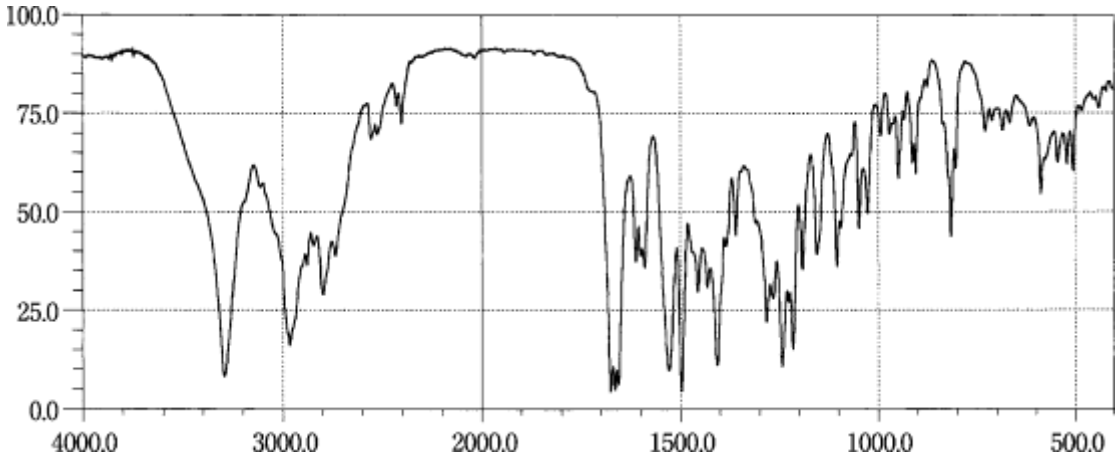


Fig. 3: FTIR of Caspofungin+ polyethylene glycol.

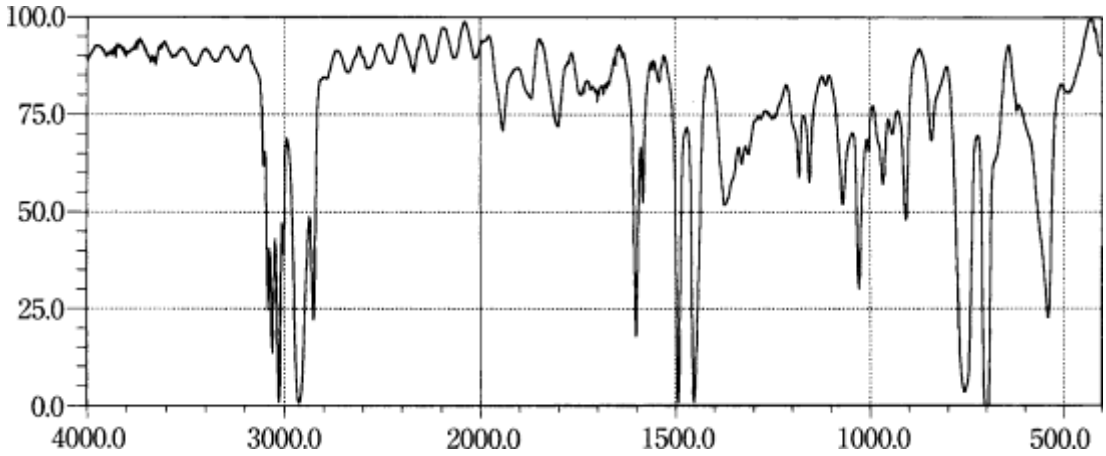


Fig. 4: FTIR of Caspofungin+ Tween 80.

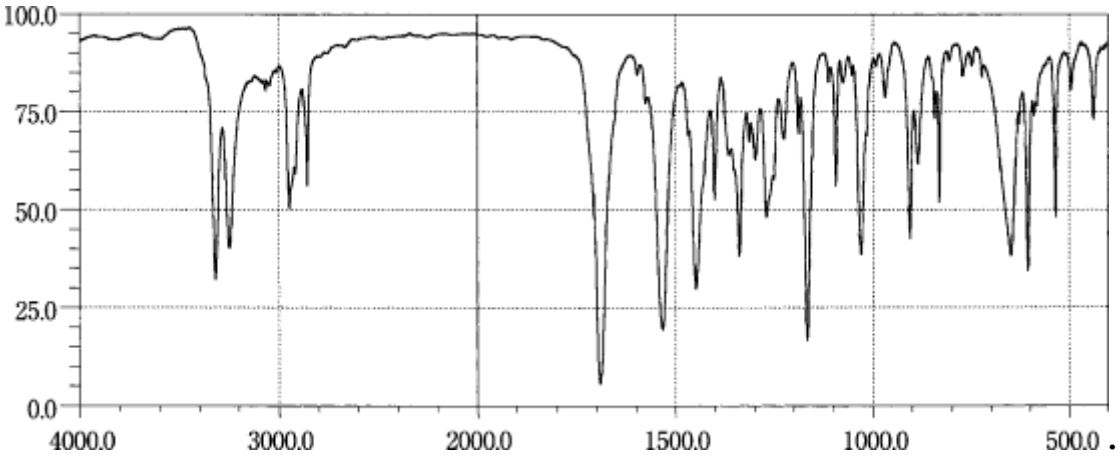


Fig. 5: FTIR of Caspofungin+ Methyl Paraben.

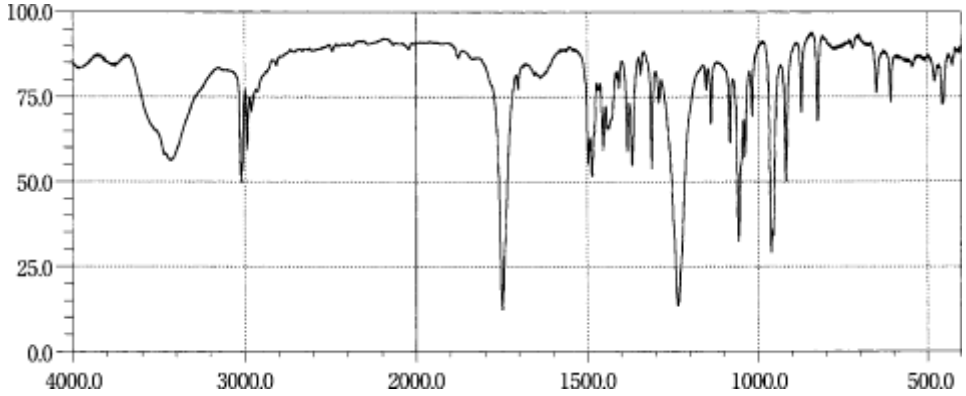


Fig. 6: FTIR of Caspofungin+ Propyl Paraben.

- **Standard calibration curve**

Table 2: Standard calibration curve of Caspofungin.

Conc. (µg/ml)	Absorbance (236nm)
2µg/ml	0.18
4µg/ml	0.34
6µg/ml	0.48
8µg/ml	0.66
10µg/ml	0.82
12µg/ml	0.95

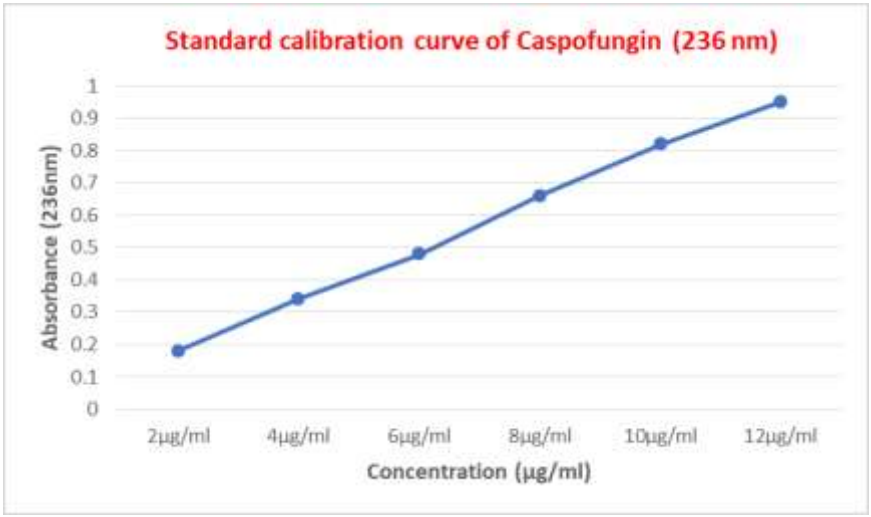


Fig. 7: Standard calibration curve at pH 7.4.

Evaluation of formulations

Physical appearances

In context to characterize total 3 forms of emulgels (F1-F3) were prepared and evaluated for their physical appearance i.e., colour, consistency and phase separation. It showed that emulgels were found as white in colour, translucent in appearance. These formulations exhibited an optimum consistency with absent phase separation.

Table 3: Physical appearance of emulgels.

Emulgel	Colour	Consistency	Phase separation
F1	White, Translucent	Optimum	Absent
F2	White, Translucent	Optimum	Absent
F3	White, Translucent	Optimum	Absent

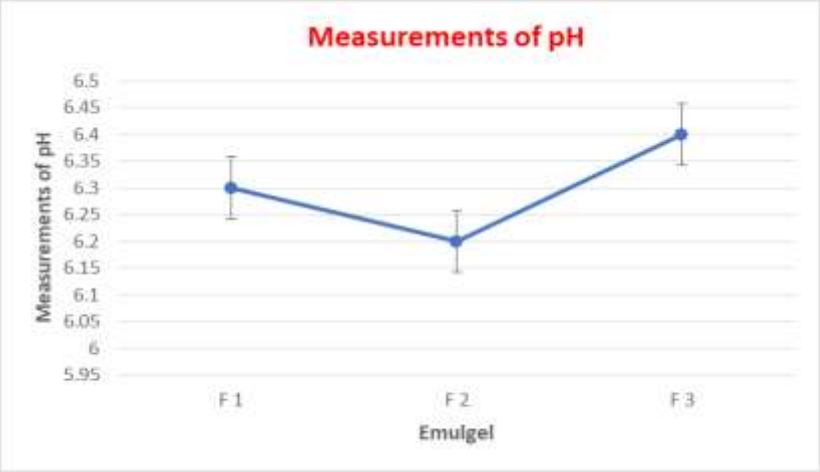
Measurements of pH

The pH level was determined to maximize solubility and absorption. pH was estimated as 6.3±0.2 in F1. While pH for F2 and F3 was measured as 6.2±0.1 and 6.4±0.3, respectively. Therefore, all the formulations showed pH range in acid environment for better solubility profile.

Table 4. Measurements of pH

Emulgel	pH range
F 1	6.3±0.2
F 2	6.2±0.1
F 3	6.4±0.3

Data were given in Mean ±S.D.; n=3



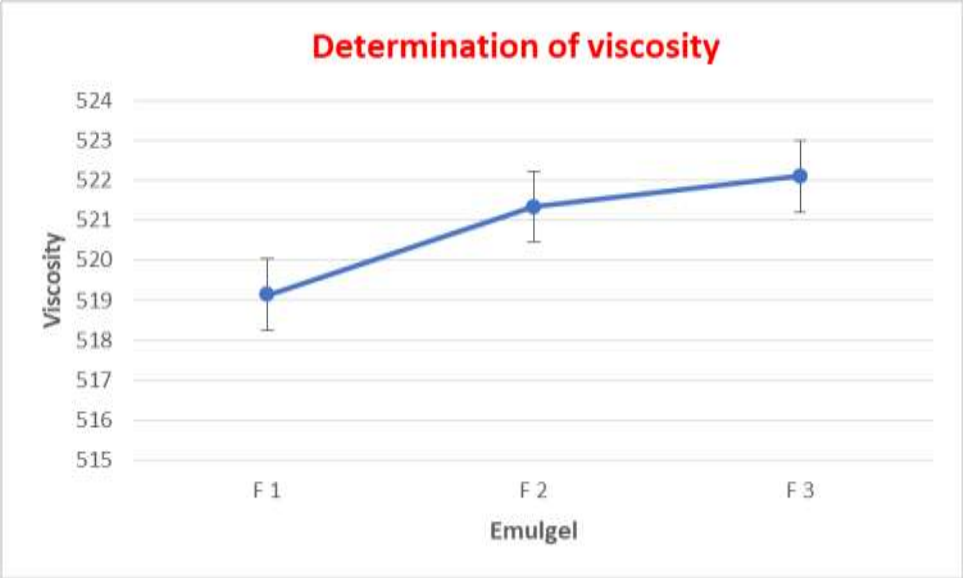
Determination of viscosity

Viscosity is the quality factor of every emulgel that assures about its consistency and uniformity. It facilitates the flowability of the formulation and in turn better availability. The lowest viscosity was estimated in F1 as 519.14±0.12. However, formulation F2 showed the viscosity as 521.34±0.18. While, F3 has shown highest viscosity as 522.10±0.23. All the preparations were demonstrated viscosity under normal standard deviation range.

High viscosity exhibits a better adhere and absorption property as shown in following table-

Table 5: Determination of viscosity.

Emulgel	Viscosity
F1	519.14±0.12
F2	521.34±0.18
F3	522.10±0.23

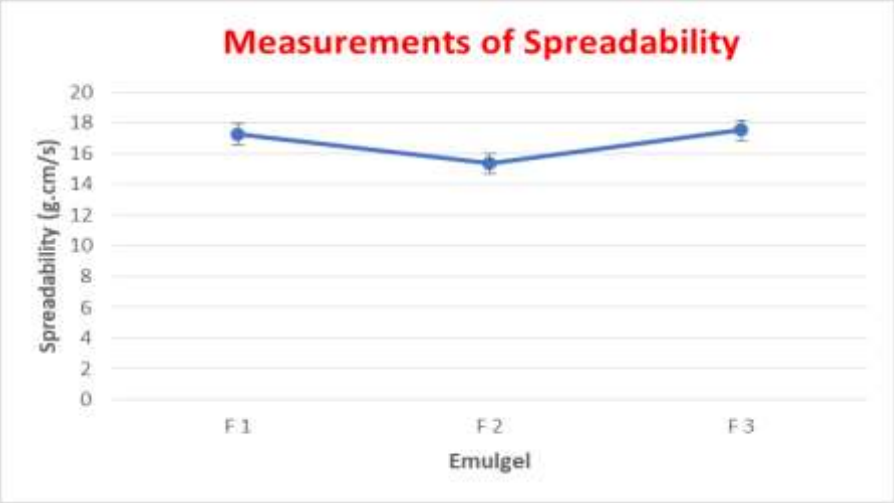


Spreadability

Spreadability defines the absorption capacity through better uniformity of contents. The spreadability data showed a specific flowability of the formulations developed. Emulgel (F1) demonstrated highest spreadability as 17.25±0.29 g.cm/s. When observed F2 and F3 were shown spreadability as 15.34±0.14g.cm/s and 17.52±0.23g.cm/s, respectively.

Table 6: Measurements of Spreadability.

Emulgel	Spreadability (g.cm/s)
F1	17.25±0.29
F2	15.34±0.14
F3	17.52±0.23

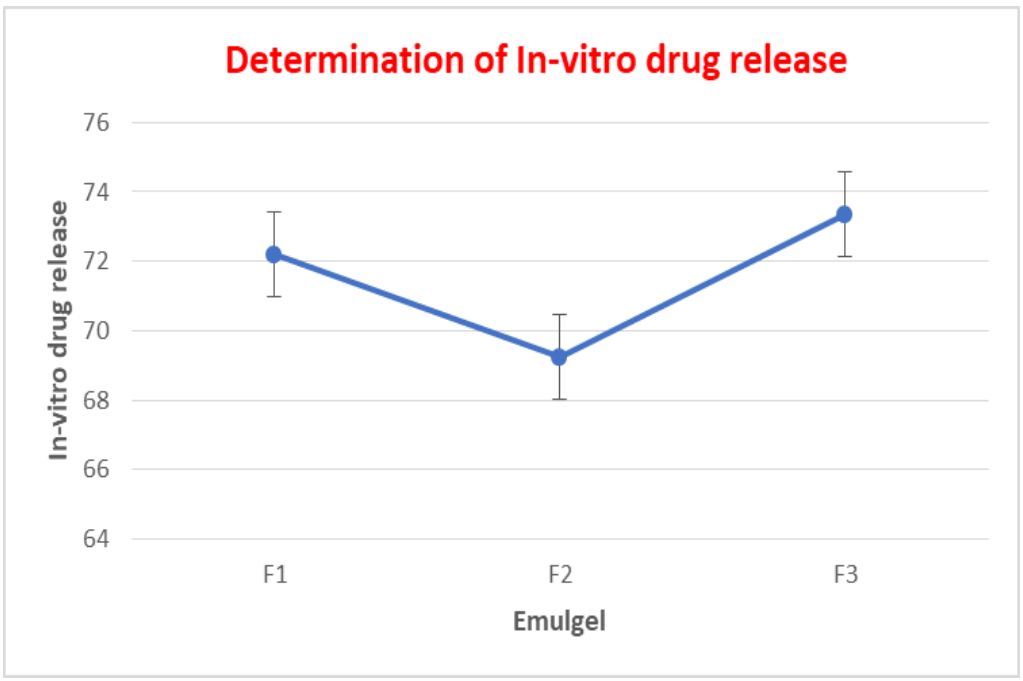


In-vitro drug release

The drug release was observed as 72.19±0.24 in emulgel (F1) and 69.24±0.21 in emulgel (F2). While, the maximum in-vitro drug release was seen in formulation F3 73.34±0.17. When compared to other formulations, *in-vitro* drug release was found optimum that indicates for its high drug release.

Table 7: Determination of In-vitro drug release.

Emulgel	In-vitro drug release
F1	72.19±0.24
F2	69.24±0.21
F3	73.34±0.17



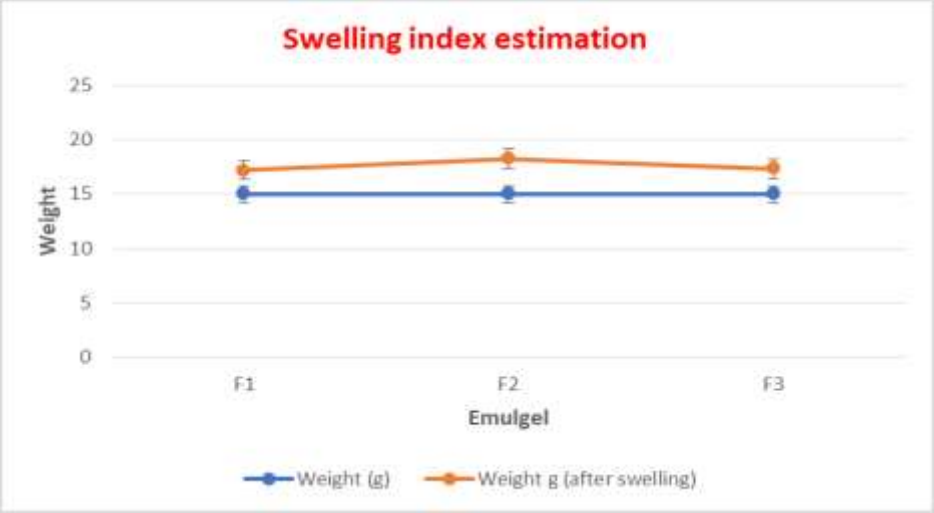
Swelling index

All the emulgels were chosen of 15g. It has demonstrated a remarkable swelling property when observed. Min. swelling index was seen in F1 as 17.18± 0.36. Whereas, almost similar swelling index was calculated in F3 as 17.36± 0.35 and F2 as 18.24± 0.31. The swelling index exhibits the concentration of polymers used for the formulation of emugel. It holds the formulations from creaming or peeling off.

Swelling power shown in below table-

Table 8: Swelling index estimation.

Emulgel	Weight (g)	Weight g (after swelling)
F1	15	17.18± 0.36
F2	15	18.24± 0.31
F3	15	17.36± 0.35



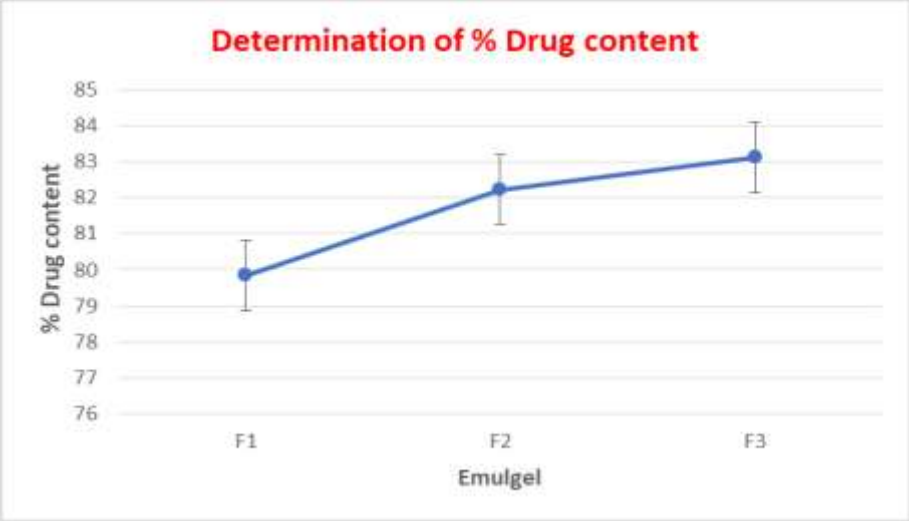
Determination of % Drug content

The prepared emulgels showed excellent % drug content in terms of better flowability or rheological properties and concentration of drug. Emulgel F1 exhibited % drug content as 78.35±0.35% that was minimum. Whereas F2 and F3 were shown increased % drug content as 82.23±0.18% and 83.12±0.26%, respectively.

Below table depicts the % drug content-

Table 9: Determination of % Drug content.

Emulgel	% Drug content
F1	79.85±0.35
F2	82.23±0.18
F3	83.12±0.26

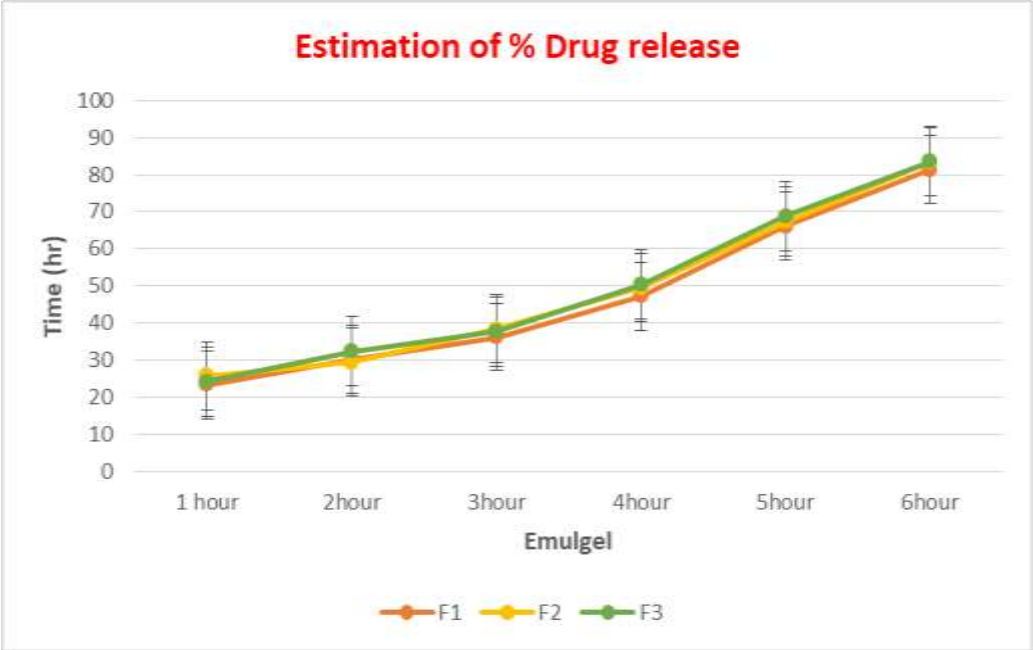


Determination % Drug release

After 6 hours, the % drug release from formulations F1, F2, and F3 was 81.31±0.11, 82.35±0.64, and 83.20±0.33, respectively. The drug release was observed identical among the 3 formulations tested. It was found in ascending order i.e., drug release increases as time increase.

Table 10: Estimation of % Drug Release.

Emulgel	% Drug release (hr)					
	1	2	3	4	5	6
F1	23.24±0.1	30.2±0.11	36.21±0.33	47.23±0.12	66.14±0.13	81.31±0.11
F2	25.72±0.2	29.45±0.22	38.40±0.22	49.42±0.32	67.33±0.17	83.35±0.64
F3	24.20±0.1	32.39±0.18	37.67±0.13	50.26±0.11	68.84±0.20	83.60±0.33

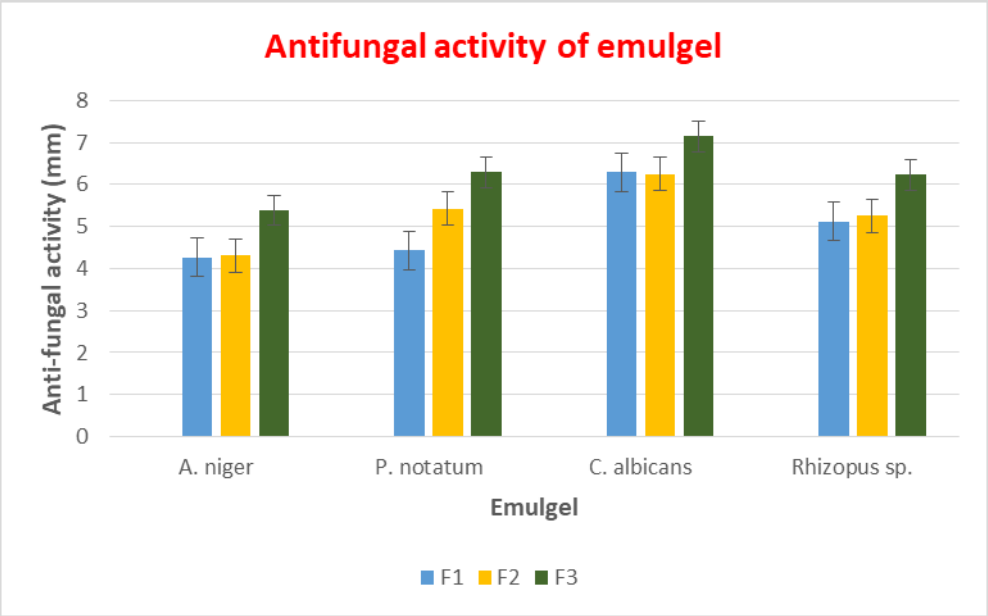


5.4 Screening of anti-fungal activity

Different 4 strains species of fungus i.e., *A. niger*, *P. notatum*, *C. albicans and Rhizopus species* were included for evaluation of anti-fungal activity. In F3, antifungal activity was recorded as 5.39mm, 6.29mm, 7.14mm, and 6.23mm in *A. niger*, *P. notatum*, *C. albicans and Rhizopus species*, respectively. It can be said that Caspofungin has more anti-fungal potential on *C. albicans* as compared to *A. niger*. It might be due to destruction of cell wall and/ nucleic acid of fungal strains.

Table 11: Antifungal activity of emulgel.

Emulgel	Anti-fungal activity (mm)			
	A. niger	*P. notatum*	*C. albicans*	*Rhizopus sp.*
F1	4.26	4.43	6.29	5.12
F2	4.31	5.43	6.24	5.26
F3	5.39	6.29	7.14	6.23



Formulation scientists need to find a way to distribute hydrophobic medicines—which are notoriously difficult to dissolve in water- in order to increase therapeutic bioavailability. Since forty percent of pharmaceuticals are hydrophobic, it is challenging for the body to absorb them. Among the several topical formulation processes, emulgel has been found to be especially significant in improving the topical dispersion of hydrophobic medications. Because gel contains an emulsion, it has a dual control release mechanism. In addition, problems like phase separation and creaming are resolved and the emulsion's stability is improved. The primary problem with emulgel is drug permeability because to its large particle size; however, by using the NEG technique, this can be resolved by adding

nanoemulsion to the gel foundation. This work is a component of the New Drug Delivery System project, which attempts to enhance the new method of regularly applying a loaded topical emulgel containing capsaicin to the skin. Analgesic: meant to relieve pain. If it could be dosed locally, maintained for a long time with few detrimental effects on the system, it would have a major impact on people's feelings of pain and inflammation.

CONCLUSION

In conclusion, there is no doubt that emulgels of Caspofungin, with their superior therapeutic potential and less patient problems, will be the most sought-after dosage form. It would be a fantastic first step toward allopathic externally applied treatments that can help millions of people live more comfortably. It may also be determined whether the costs associated with its mass production are manageable. It would help the emulgel's stability in the formulation a lot. As a result, you'll need to take less Caspofungin to achieve the same effect.

It suggests, to isolate the significant constituent that are effective in the treatment of various topics illnesses like inflammation, pruritis etc.

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