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Development Of A Caspofungin-Loaded Emulgel: Physicochemical Characterization And Antifungal Efficacy Assessment Using FTIR

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Abstract

Background: *Caspofungin, an echinocandin antifungal, has limited topical application due to poor solubility and skin permeability. To overcome these limitations, a Caspofungin-loaded emulgel was developed, aiming to enhance local drug delivery and improve antifungal efficacy against skin infections.*

Method: *A stable emulgel formulation was developed using Carbopol 940 as the gelling agent and liquid paraffin as the oil phase. Nine batches (F1–F9) were prepared by varying concentrations of Carbopol and paraffin, optimized through a Central Composite Design (CCD). Compatibility between drug and excipients was confirmed using FTIR. Evaluation included pH, viscosity, spreadability, drug content, and in-vitro drug release via Franz diffusion cell.*

Research: *All formulations were physicochemically stable. Batch F7 exhibited the most promising results with 96% drug content and 97.3% drug release. Viscosity and spreadability were within acceptable limits, ensuring ease of application. Statistical analysis showed Carbopol 940 significantly influenced drug content, while liquid paraffin had a major effect on drug release. Response surface and contour plots validated these interactions.*

Conclusion: *The optimized emulgel formulation demonstrated excellent stability, uniform drug distribution, and enhanced in-vitro drug release. Caspofungin emulgel represents a promising topical delivery system for antifungal treatment, combining improved skin permeability and patient compliance.*

1. Background:

Drug and Polymer Compatibility Study of Caspofungin by FT-IR

In pharmaceutical formulation one of the requirements for the selection of suitable excipient is compatibility. Therefore, In the present work for confirmation of any possible chemical interaction between the Caspofungin and Carbopol 940 polymer was carried out using Fourier transformed infrared (FT-IR) spectrophotometer (using perkin elmer). IR by potassium pellet method was carried out on pure substance i.e., drug and their physical mixture. Transparent pellet formed by compressing under 15 tones' pressure in a hydraulic press. The pellet was scanned from 4000 to 400 cm^{-1} in a spectrophotometer. The spectrum of physical mixture was compared with the original spectra to determine any possible molecular interactions between the drug and polymer. Fourier Transformer Infrared Spectroscopy (FTIR) analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample. The observation of vibration spectrum of encapsulated drug by evaluates the kind of interaction occurring between the drug and polymer.

2. Method:

Method of preparation of emulgels

Different formulation was prepared by using various ratio of gelling agent and penetration enhancer. The preparation of emulgel was same for all formulation batches.

"Gel phase in the prepared formulation was formulated by mixing Carbopol 940 in distilled water with

constant stirring at moderate speed by using magnetic stirrer, further, formulation or emulgels pH was adjusted by to 6 using tri ethanolamine and the oily phase in the formulation was prepared by mixing tween 20 in light paraffin oil and aqueous or water containing phase was dissolved by mixing tween 20 in distilled water". Further, "Methyl Paraben and Propyl Paraben preservatives were added in propylene glycol and drug was dissolved in dimethyl sulfoxide and the drug solution and preservative solution mixed with aqueous solution, both aqueous and oily solution is separately heated to temperature 70-80^{0}C, then oily phase was dispersed in aqueous phase with constant continuous stirring until it got cooled at room temperature and finally, the obtained emulsion was mixed with the gel in a 1:1 ratio with gentle stirring to obtain the emulgel". The composition of different formulations has been discussed in below table.

Table 1: Composition of different formulation batches (%w/w).

Ingredient(%w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Caspofungin	1	1	1	1	1	1	1	1	1
Carbopol 940	0.5	2.5	0.5	0.5	2.5	4.5	2.5	4.5	4.5
Liquid paraffin	9.0	1.0	1.0	5.0	9.0	9.0	5.0	5.0	1.0
Tween 20	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Span 20	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Propylene glycol	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DMSO	5	5	5	5	5	5	5	5	5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Optimization of Topical emulgel:

To "statistically optimize the formulation parameters of the drug content and drug release of gel, a central composite design with two components, 2 levels, and nine runs was chosen and the response surface model and polynomial models were investigated utilizing two factors and two-level designs, allowing for the optimization of a process with a small number of experimental runs, in which a set of locations at the midpoint of each edge and the repeated centre point of the multidimensional cube make up the experimental design". This experimental design yielded the following polynomial equation:

$$Y_1 = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_1X_2 + \beta_4X_1^2 + \beta_5X_2^2 \text{......} \quad eq^n 1$$

Where Y_1 is the dependent variables, b0 is the intercept

"X_1, X_2, are the independent variables that were selected from the Preliminary experiments, Independent variables studied were carbapol 940 (X_1), liquid paraffin (X_2), and the dependent variables were drug content (Y_1) and drug release (Y_2),the range of independent variables under study is shown along with their low, medium, and high levels, which were selected based on the result from preliminary experiments".

Experimental Design

A 2^3rd "the effect of independent factors I concentration gelling agent (X1) and (ii) concentration liquid paraffin (X2) and dependent variables (conc Emulsifying agent) was investigated using a central composite design (X3), further, determination of drug content (Y1) and percent drug release after 1 hour (Y2)".

Characterization study of Emulgel

Physical Properties:

Physicochemical parameters such as "colour, odour, and consistency were examinedin the Caspofungin-containing emulgel formulations".

Determination of Drug Content:

The concentration of Caspofungin in emulgel was determined by sonicating a known amount of emulgel in solvent (ethanol). A UV spectrophotometer was used to determine absorbance after an appropriate dilution at 272 nm.

FTIR Study:

The test was conducted "to identify and verify the integrity of functional groups of API utilised in the

preparation of emulgel, Caspofungin, for example A Fourier transform infrared spectrophotometer was used to evaluate the FTIR of pure medication and a physical mixture of formulation ingredients in the optimal batch (BRUKER) and The physical mixture contained the same amount of each formulation ingredient as the optimised batch, further, separately, the pure drug and physical mixture were combined with IR grade KBr and finally, this mixture was then scanned over a 4000to 650 cm^{-1}wavenumber range". The scans were examined for the existence of a drug's main peaks.

In-Vitro Drug Release Studies:

A modified "Franz diffusion (FD) cell was used in the in-vitro drug release tests, firstly the formulation was applied to a dialysis membrane sandwiched between the donor and receptor compartments of the Franz diffusion cell" (which had previously been soaked in phosphate buffer pH 3.2 for 24 hours). By putting the cell in the water bath, the temperature was kept at 370.2°C. This "entire assembly was placed on a magnetic stirrer, and the solution was continually stirred at 50 rpm using a magnetic bead, after adequate dilutions, the samples (2ml) were withdrawn at a sufficient time interval and examined for drug content using a UV visible spectrophotometer at 272 nm". The percent drug release was estimated using UV Spectroscopy at 272 nm.

pH:

We measured the pH of the created emulgel mixtures using a digital pH metre. 100 millilitres of distilled water were used to dissolve 1 gram of emulgel, and the mixture was let to sit for 2 hours.

Rheological Study:

The "viscosity was measured with a cone and plate viscometer with spindle 7 (Brookfield rheometer) and the viscosity-to-be-measured formulation was placed in a beaker and allowed to settle for 30 minutes, before the measurement, the temperature was set to the assay temperature (250°C) and the spindle was lowered perpendicularlyinto the centre of the emulgel, making sure it did not touch the jar's bottom, and rotated at 50 rpm for 10 minutes, further, the spindle was moved up and down, resulting in viscosities at various locations along the path". The viscosity of the gel was calculated as the average of values made over a 10-minute period.

Spreadability

A pulley is fastened to one end of a wooden block that makes up the device. Using the'Slip' and 'Drag' characteristics of the emulgel, the spreading coefficient was computed. On the wood block, a glass slide was set in the ground. On this ground slide, there was an extra 1 g of emulgel. Between this one and the emulgel was a second glass slide that was the same size as the fixed ground slide. The hook is included with the second glass slide. To remove air from the area between the two slides and produce a uniform layer of emulgel, a 100 g weight was placed on top of them for five minutes. The measured weight (35g) was dropped into the pan fastened to the pulley using a hook."Time in seconds it takes for two slides to separate" from the emulgel and be put in between them under the influence of a specific force. The spreadability improves as the time it takes to separate two slides decreases.

The formula is used to calculate it, in which,

S = ML / T

(Where M = wt tied to upperslide, L = length of glass slides, and T = time taken to separate the slides)".

3. RESULT:

FTIR Study

The FTIR spectrum of Caspofungin is shown in figure. It showed that, functional group band frequencies of Caspofungin were in resemblance to the reported range of standard Caspofungin that authenticated that the obtained sample of Caspofungin was pure.

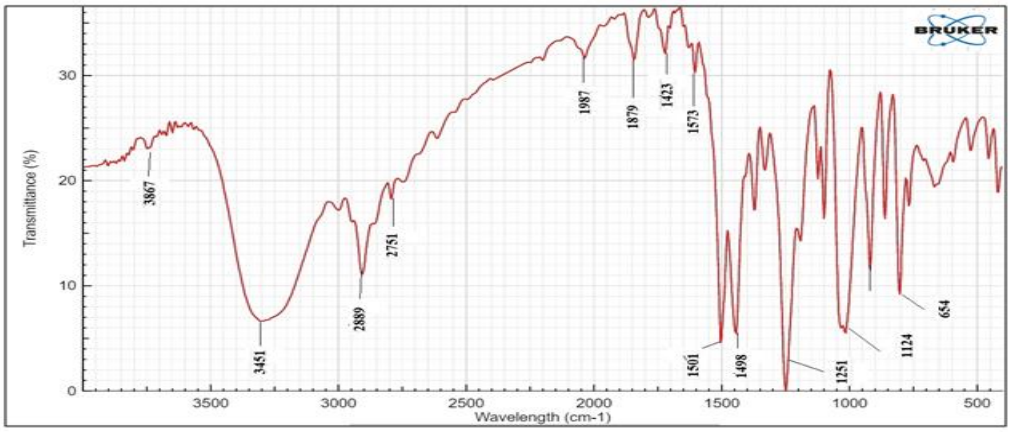

Figure 1: FTIR of Caspofungin

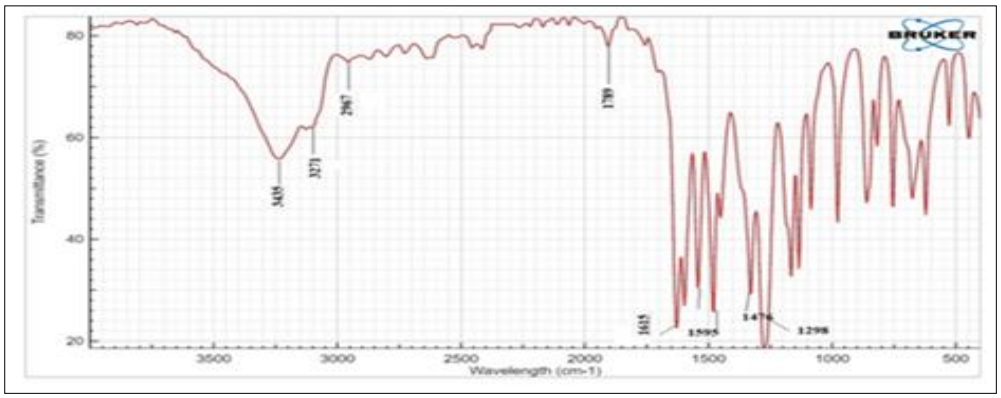

Figure 2: FTIR of Drug and excipients Compatibility study

Evaluation of Emulgel:

Physical stability

The physical properties (colour, appearance, homogeneity, and phase separation) of optimized Caspofungin were observed visually and by touching to assess any changes. The homogeneity and texture of Caspofungin were assessed by pressing a small quantity of Caspofungin between the thumb and index finger. The optimized Caspofungin showed yellow color, and pleasing and graceful (transparent or elegant) appearance. Moreover, the Caspofungin was found to be homogeneous in character or appearance (homogeneous texture) when pressed between the thumb and index finger.

Drug content determination by UV

1gm of emulgel was dissolved in 10 ml of ethanol. "The volumetric flask was kept for1 hour and shaken well in a shaker to mix it properly and the solution was passed through the filter paper and filtered, further, withdrawn 1ml was diluted up to 10 ml with phosphate buffer pH 3.2 the absorbance was measured spectrophotometrically at 272 nm and finally the drug content was determined using a standard plot".

Table 2:Drug content of percentage value

Sr no.	Formulation code	% drug content
1	F1	95±1.52
2	F2	92±1.21
3	F3	94±1.89
4	F4	93±1.59
5	F5	89±1.62
6	F6	91±1.58

7	**F7**	**96±1.23**
8	F8	92±1.57
9	F9	93±1.58

The average drug content of Caspofungin gel was found to be in percentage. Drug contentvalues were almost uniform in all F1-F9 formulations as mentioned in table. formulation F7 shows very good drug content.

In-Vitro Drug Release Studies

Drug release studies were performed in Franz diffusion cell applied on dialysis membrane which is used in diffusion media of phosphate buffer solution 3.2 withdrawn 2ml sample diluted in PBS 7.4 at 10 min time interval absorbance measured in determining λ max at 272 nm by UV spectrophotometer in all formulation. The in vitro test was performed to ensure the uniform and accurate permeability of the drug. a good drug permeability was observed among all emulgel formulations and was found to be 97.3

Table 3 : Drug release of formulation F1-F9

FORMULATION CODE	% DRUG RELEASE
F1	70.33±1.61
F2	78.22±0.66
F3	82.44±1.96
F4	79.44±1.37
F5	82.55±0.89
F6	89.23±1.68
F7	97.3±1.49
F8	89.44±1.96
F9	89.34±0.57

Optimization of Caspofungin Emulgel

Effect of formulation variables on drug content:

"Statistical Analysis of the central composite design batches was performed by multiple regression analysis using Design of Experiment (version 10) software, resultsof ANOVA shown below table.

Response 1: Drug Content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	35.44	5	7.09	191.40	0.0006	significant
A- Carbopol 940	6.00	1	6.00	162.00	0.0010	
B-Liquid Paraffin	0.0000	1	0.0000	0.0000	1.0000	
AB	1.0000	1	1.0000	27.00	0.0138	
A^2	14.22	1	14.22	384.00	0.0003	
B^2	14.22	1	14.22	384.00	0.0003	
Residual	0.1111	3	0.0370			
Cor Total	35.56	8				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 191.40 implies the model is significant. There is only a 0.06% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	0.1925		**R^2**	0.9969
Mean	92.78		**Adjusted R^2**	0.9917
C.V. %	0.2074		**Predicted R^2**	0.9768
			Adeq Precision	43.4871

The ANOVA for the dependent variables demonstrates that "the model was significant for all response variables and the effect is like, the amount of Carbopol 940 and liquid paraffin were found to be significant, along with its quadratic and interaction terms for all the dependent variables".

Analysis of Contour plot & response surface model:

Figure displays 2D contour plots, which are excellent for examining how a component interacts with the responses. These kinds of plots are helpful for examining the simultaneous impact of two variables on the answer and the overall given figure. The effects of X1 (carbapol) and X2 (liquid paraffin) with their non-interaction on drug content at a fixed or level X2 are all linear up to a specified range.

The counterplot makes it clear that the formulation benefits from the increased amount of both X1 and X2.

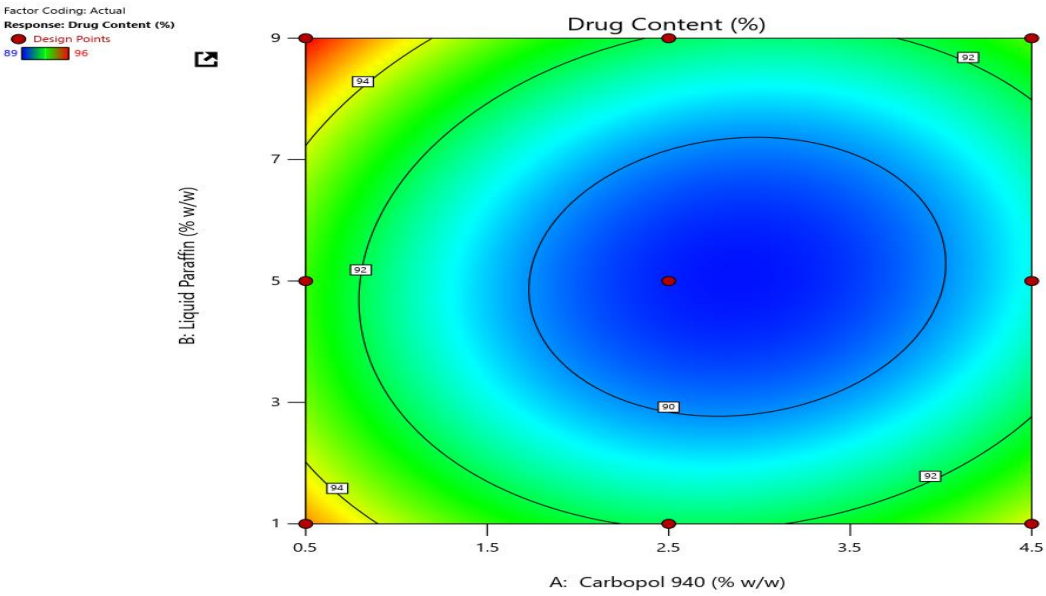

Fig. 3: Contour plot of drug content

Response surface of 3D plot

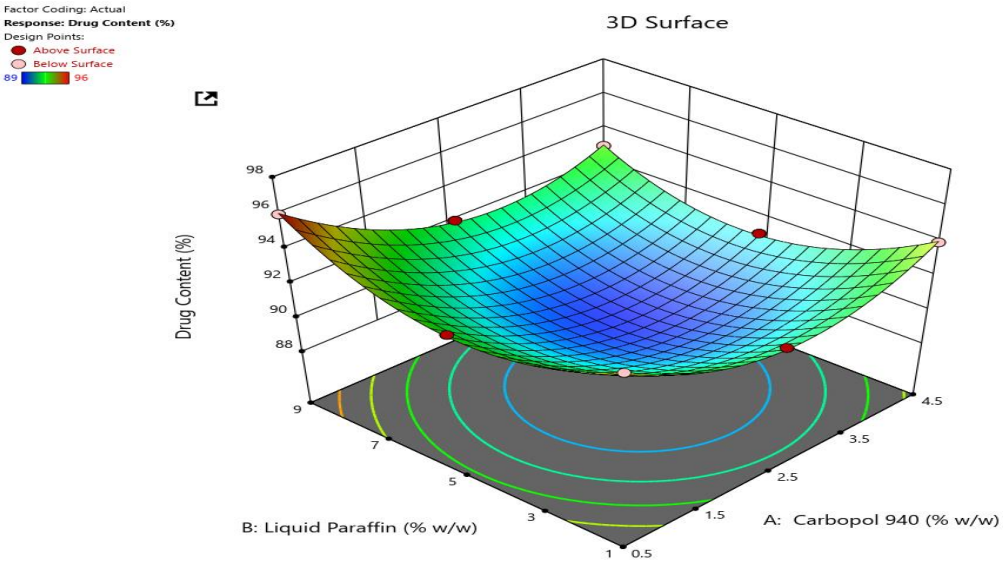

Fig. 4 : Response surface plot (3D) of drug content

Figure shows three-dimensional response surface plots, which are highly helpful for examining how different factors interact to affect responses. These charts are highly helpful for forecasting the outcomes of experiments. These diagrams are highly helpful for understanding the link between the dependent and independent variables, as well as the simultaneous effects of two components.

Effect of formulation variables on in vitro drug release

The "ANOVA for the dependent variables demonstrates that the model was significant for all response variables and the effect is like, the amount of carbapol and liquid paraffin were found to be significant, along with its quadratic and interaction terms for all the dependent variables".

Response 2: In-Vitro Drug Release

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	476.39	5	95.28	9.22	0.0485	Significant
A- Carbopol 940	32.39	1	32.39	3.14	0.1747	Not significant
B-Liquid Paraffin	338.85	1	338.85	32.81	0.0106	Highly Significant
AB	100.70	1	100.70	9.75	0.0524	Marginal
A^2	3.26	1	3.26	0.3156	0.6135	Not Significant
B^2	1.19	1	1.19	0.1153	0.7566	Not Significant
Residual	30.99	3	10.33			
Cor Total	507.38	8				

Factor coding is **coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 9.22 implies the model is significant. There is only a 4.85% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant.

Fit Statistics

Std. Dev.	3.21		**R^2**	0.9389
Mean	84.25		**Adjusted R^2**	0.8371
C.V. %	3.81		**Predicted R^2**	0.2579
			Adeq Precision	9.5519

Analysis of Contour plot & response surface model:

Figure displays 2D contour plots, which are excellent for examining how a component interacts with the responses. These kinds of plots are helpful for examining the simultaneous impact of two variables on the answer and the overall given figure. The effects of X1 (carbapol) and X2 (liquid paraffin) with their non-interaction on drug content at a fixed or level X2 are all linear up to a specified range

The counterplot makes it clear that the formulation benefits from the increased amount of both X1 and X2.

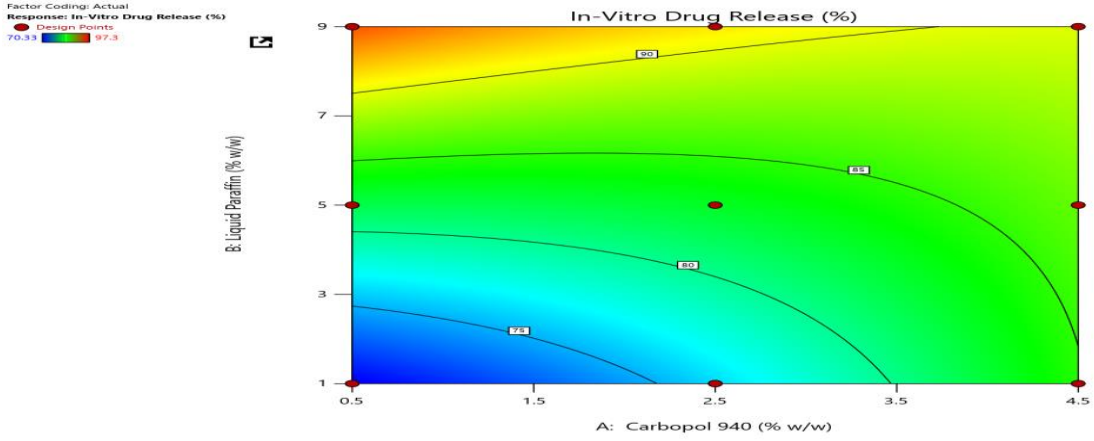

Fig. 5: Contour plot of drug release

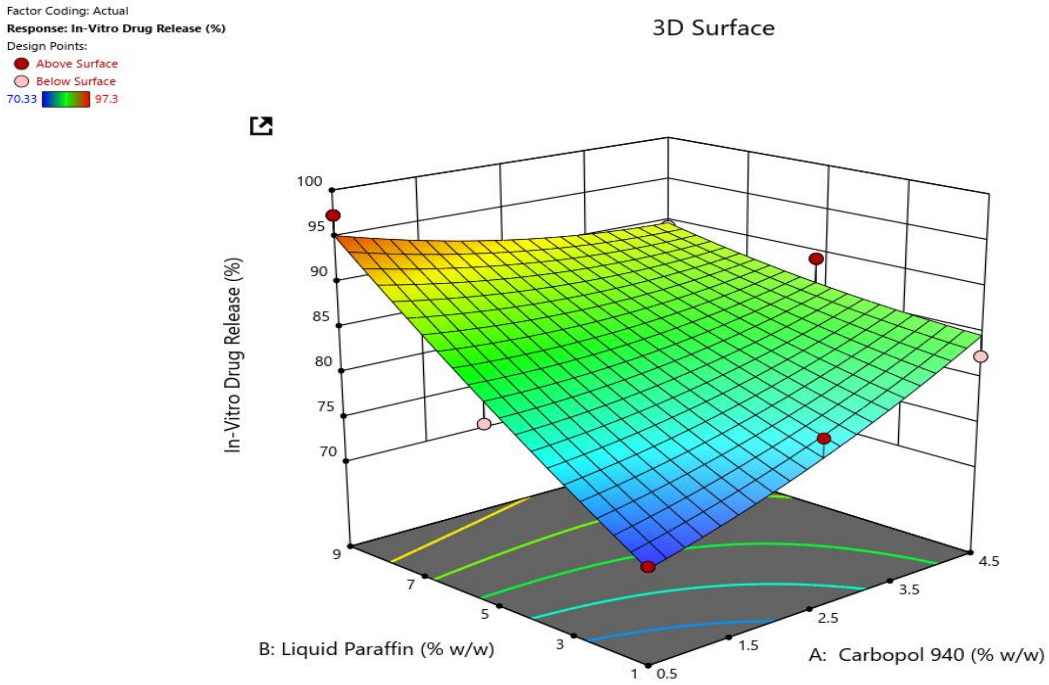

Fig. 6: Response Surface Plot of drug release

Measurement of pH:

"The pH of developed emulgel formulations was determined using a digital pH meter,first, 1gm of emulgel was dissolved in 100 ml distilled water and kept aside for two hours and the measurement of pH of each formulation was done in triplicate and average values are calculated shown in table".

Table 4 : pH values of formulation F1-F8

Sr. No.	Formulation code	pH
1	F 1	6.20±0.36
2	F 2	6.50±0.35
3	F3	6.34±0.18
4	F4	6.64±0.16
5	F5	6.10±0.44
6	F6	6.03±0.37
7	F7	6.15±0.53
8	F8	6.69±0.29
9	F9	6.12±0.16

Viscosity Measurement

Statistically, The maximum viscosity was observed in F7, that contains CP 940 and LP, this could be explained by the higher molecular weight of the CP 940 in comparison with the other two polymer and also refer to the addition of the neutralizing agent Triethanolamine in CP 940 formulas. In gel systems,

consistency depends on the ratio of solid fraction, which produces structure, to liquid fraction. The profiles showed that as the share stress increased, the normally arranged molecules align their long axes in direction of flow orientation reduce the internal resistance of material and hence decrease viscosity .The results showed that within each type of polymer the viscosity increased as the concentration of polymer increased. Most of the prepared formulations are of good acceptable rheological profile ranged mentioned in many literatures, which is 7100-83144 cps. However, the attentiveness of viscosity increases with the understanding of the extent of increased viscosity on drug release retardation and stability of formulas prepared.

Spreadability:

Spreadability is a term expressed to denote "the extent of the area to which the topicalapplication spreads on application to the skin on the affected parts and the efficacy ofa topical therapy depends on the patient spreading the drug formulation in an even layer to administer a standard dose, hence, the determination of spreadability is very important in evaluating topical application characteristics". The spreadability of the Caspofungin emulgel was found to be 21.80±0.89 g.cm/sec which indicates it has good spreadability. it is calculated using formula and results have been reported in graph.

Table 5: Spreadability values of formulation F1-F8

Sr no.	Formulation code	Spreadability g.cm/sec
1	F1	2.20±0.48
2	F2	1.36±0.5
3	F3	1.40±0.92
4	F4	2.32±1.11
5	F5	2.18±1.68
6	F6	1.59±1.66
7	F7	2.80±0.89
8	F8	2.62±1.26
9	F9	1.73±1.11

4. CONCLUSION:

The present study successfully developed and evaluated a stable Caspofungin-loaded emulgel for the topical treatment of fungal infections. Drug-polymer compatibility was confirmed through FTIR analysis, indicating no chemical interactions between Caspofungin and Carbopol 940. Using a Central Composite Design (CCD), nine formulations were prepared and optimized for drug content and drug release. Among these, formulation F7 exhibited superior performance with the highest drug content (96%) and drug release (97.3%). The formulations were evaluated for pH, viscosity, spreadability, and in-vitro drug release. The optimized batch demonstrated acceptable pH (~6.15), desirable viscosity, and excellent spreadability (2.80 ± 0.89 g·cm/sec), indicating ease of application and patient compliance. Rheological analysis confirmed good gel structure and stability.

Statistical analysis validated the influence of formulation variables—Carbopol 940 and liquid paraffin—on drug content and release. The model was found significant with a high R^2 value (>0.99), confirming the robustness of the design approach.

Overall, the optimized Caspofungin emulgel formulation offers a promising alternative for effective topical antifungal therapy, combining controlled drug release, enhanced skin permeability, and patient-friendly application characteristics.

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