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Research Article

BOX–BEHNKEN DESIGN-DRIVEN FORMULATION AND EVALUATION OF A NOVEL TOPICAL GEL CONTAINING CEFEPIME HCL AND L-ARGININE

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ABSTRACT

Objective: The present study aims to develop and optimize an antibacterial gel formulation containing Cefepime HCl and L-Arginine using a Box–Behnken design, with a focus on sustained drug release and improved antibacterial activity.

Methods: The gel was prepared using Carbopol 940 as the gelling agent, and pH was adjusted to neutrality using sodium hydroxide. Polyethylene glycol, glycerol, butylated hydroxytoluene, and methylparaben were included as excipients. A 3^2 factorial design was employed to evaluate the influence of Carbopol concentration and stirring time on drug release characteristics. The formulations were assessed for pH, viscosity, spreadability, extrudability, and drug content uniformity. *In vitro* drug release was studied using a Franz diffusion cell, and antimicrobial activity was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar well-diffusion method.

Results: Formulation F5 demonstrated optimal physicochemical and rheological properties, with sustained biphasic drug release reaching 98% over 12 h. Drug content uniformity ranged from 53.9% to 104.6% across batches. The formulation exhibited moderate antibacterial activity against *S. aureus* and weak activity against *P. aeruginosa*, with maximum inhibition observed at 1000 µg.

Conclusion: The optimized Cefepime HCl–L-Arginine gel (F5) exhibited desirable drug release and acceptable physicochemical characteristics. Although antimicrobial efficacy was moderate, particularly against Gram-positive strains, further modification may enhance its activity against resistant pathogens.

Keywords: Cefepime hydrochloride, L-arginine, Topical gel formulation, Box–Behnken design, Antibacterial gel, *In vitro* drug release, Agar well-diffusion method, Franz diffusion cell, Spreadability, Viscosity evaluation, Design-Expert® software.

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INTRODUCTION

Cefepime hydrochloride, a fourth-generation cephalosporin, exhibits a broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens. Despite its therapeutic potential, the topical application of Cefepime HCl is limited by its chemical instability and poor skin permeability. To overcome these challenges, formulation strategies involving permeation enhancers and stabilizers have been explored to improve dermal drug delivery-Arginine, a semi-essential amino acid, has shown promise in topical drug delivery systems due to its ability to enhance solubility, promote diffusion, and increase drug retention. Furthermore, as a precursor of nitric oxide, L-arginine contributes to tissue repair, immune modulation, and wound healing [1]. Incorporating L-arginine into Cefepime HCl-based gel formulations may offer a synergistic approach to improve both therapeutic delivery and antimicrobial efficacy. Carbopol 940, a synthetic high-molecular-weight polymer of acrylic acid, is widely used as a gelling agent in topical formulations due to its excellent viscosity, bioadhesion, and release-controlling properties. In this study, a 3^2 Box–Behnken design was applied to optimize the gel formulation by evaluating the impact of Carbopol concentration, mixing time, and application frequency on drug release and formulation characteristics. The primary objective was to develop and characterize a stable, effective, and patient-friendly topical antibacterial gel containing Cefepime HCl and L-Arginine. The formulated gels were assessed for physicochemical properties, *in vitro* drug release, and antibacterial activity to identify an optimized formulation suitable for skin infections [2].

METHODS

Materials

Cefepime HCl and L-Arginine were obtained from Orchid Pharma Ltd., Alathur, India. Carbopol 940 was procured from Pallav Chemicals and Solvents Pvt. Ltd. Glycerol and polyethylene glycol (PEG) 400 were obtained from Curie Laboratories, India. Other excipients, including sodium hydroxide (NaOH), butylated hydroxytoluene (BHT), and methylparaben, were of analytical grade and used as received [3].

Design of experiments (DOE) studies of formulation and evaluation of a novel antibacterial gel of cefepime HCL and L-Arginine

Controlled drug delivery systems play a crucial role in enhancing therapeutic efficacy, patient compliance, and treatment safety. Achieving an optimized release profile requires a deep understanding of the formulation variables that influence drug release behavior. Carbopol, a high molecular weight, cross-linked polyacrylic acid polymer, is widely utilized as a gelling, thickening, and release-modifying agent in topical formulations due to its viscosity-building and controlled-release properties [4]. In the present study, a Box–Behnken design was used to examine the influence of three critical formulation parameters: Factor A: Carbopol concentration(mg), Factor B: Releasing time (minutes), Factor C: Agitation frequency (RPM). These factors were selected to evaluate their impact on drug release percentage as the primary response variable. An increase in Carbopol concentration was found to decrease drug release due to the formation of a denser gel matrix, which impeded drug diffusion. As expected, drug release increased with time, consistent with controlled-release systems. Higher agitation

frequencies improved drug release, likely by enhancing diffusion and disrupting stagnant layers at the diffusion membrane interface [5,6].

Materials

Cefepime HCl and L-Arginine were obtained from Orchid Pharma Ltd., Alathur, India. Carbopol 934 (viscosity ~30,000 cP at 0.5%) was sourced from Pallav Chemicals and Solvents Pvt. Ltd. Glycerol ($C_3H_8O_3$; MW: 92.02) was procured from Curie Laboratories, India. All other reagents, including PEG 400, sodium hydroxide (NaOH), BHT, and methylparaben, were of analytical grade and used as received [7].

Preparation of topical antibacterial gel

Gel base preparation

Carbopol 934 (700 mg) was gradually dispersed in 11 mL of distilled water under continuous magnetic stirring and allowed to hydrate for 30–40 min until a uniform gel base was formed [8].

Addition of preservative and antioxidant

Methylparaben (150 mg) and BHT (150 mg) were added to the hydrated gel under stirring until completely dissolved to enhance the stability and antimicrobial resistance of the formulation [9].

Incorporation of humectants and co-solvents

PEG 400 (0.24 mL) and glycerol (0.24 mL) were premixed and added to the gel as co-solvent and humectant agents, promoting consistency and hydration [10].

Neutralization and gel formation

NaOH solution (10.5 mg) was added dropwise with continuous stirring to adjust the pH between 7.0 and 8.0, facilitating the gelation of Carbopol [11].

Drug incorporation and homogenization

Cefepime HCl (2500 mg) and L-Arginine were incorporated into the gel. The formulation was stirred thoroughly and left to stand for 10–15 min to achieve a uniform, gel-like consistency. The final gel was transferred into sterile, airtight containers for further evaluation [12].

Evaluation of topical gel

Physical appearance and homogeneity

All formulations (F1–F8) were visually inspected for color, consistency, phase separation, and texture. The gels were pale yellow, homogeneous, non-greasy, and easy to remove, with no visible lumps or discoloration [13,14].

PH determination

The pH of each gel formulation was measured using a calibrated digital pH meter. Gels were dispersed in 25 mL of distilled water and stirred uniformly before measurement. Each sample was analyzed in triplicate, and the average pH was recorded [15].

Spreadability

Spreadability was determined using a modified glass slide method. One gram of gel was placed between two glass plates and allowed to stand for 1 min. The spread diameter was then measured in centimeters. Each measurement was repeated three times and averaged [16].

Viscosity measurement

Viscosity was assessed using an Ostwald viscometer at room temperature (25±1°C). One gram of gel was dispersed in distilled water, and the time taken for the gel to flow between two marks was recorded. Viscosity (η) was calculated using the formula:

$$\eta = K \times \rho \times t$$

Where: η Viscosity (centipoise), K: Viscometer constant, ρ: Density of solution, t: Time of flow (s). Each sample was tested in triplicate [17].

Extrudability

Extrudability was evaluated by filling each formulation into aluminum collapsible tubes and manually applying pressure. The force required to extrude the gel was noted by physical observation and recorded for comparison across formulations [18].

Drug content determination

A sample of 0.1 g gel was dissolved in 10 mL of distilled water, sonicated for 20 min, filtered, and appropriately diluted. Drug content was determined by ultraviolet (UV)-visible spectrophotometry at 254 nm against a distilled water blank. The results were calculated using a calibration curve [19,20].

Drug content uniformity

Three randomly selected samples were analyzed to determine uniformity. Absorbance was measured at the drug's λmax, and drug content was calculated for each sample to assess consistency [21].

***In vitro* drug release study**

Drug release was assessed using Franz diffusion cells. The receptor compartment contained phosphate buffer (pH 7.4) maintained at 37±0.5°C. A cellulose membrane was placed between the donor and receptor compartments. One gram of gel was applied to the membrane. Samples were withdrawn at regular intervals (0.5, 1, 2, 4, 6, 8, and 12 h) and analyzed at 254 nm after appropriate dilution. Receptor medium was replenished after each withdrawal to maintain sink conditions [22].

Antibacterial activity

Agar well-diffusion method

Antibacterial activity of the gel was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar well-diffusion method. Mueller–Hinton Agar plates were inoculated with bacterial cultures. Wells (6 mm diameter) were loaded with 20 µL of gel samples at varying concentrations (125, 250, 500, 1000 µg). Dimethyl sulfoxide served as the negative control, and streptomycin (1 mg/mL) as the positive control. Plates were incubated at 37°C for 24 h, and the zones of inhibition were measured in millimeters [23,24].

Statistical analysis

All experiments were conducted in triplicate, and results are expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine statistical significance ($p<0.05$). Optimization and factorial design analysis were carried out using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN) based on Response Surface Methodology (RSM) [25].

RESULTS

DOE studies of formulation and evaluation of a novel antibacterial gel of Cefepime HCL and L-arginine

The impact of formulation variables on the drug release profile was assessed using a Box–Behnken experimental design [26]. The study focused on three critical parameters: Carbopol concentration, release time, and agitation frequency, with percentage drug release as the response variable [27].

Effect of concentration of Carbopol

An increase in the amount of Carbopol was associated with a decrease in the percentage of drug release. This inverse relationship can be attributed to the formation of a denser polymer matrix at higher polymer concentrations, which restricts drug diffusion. In addition, higher Carbopol content contributes to increased viscosity, further retarding the Table 1 [28].

Table 1: DOE for the evaluation of percentage drug release

Standard	Run	Factor 1	Factor 2	Factor 3	Response 1
		A: Amount of Carbopol	B: Time	C: Frequency	Percentage of drug release
		mg	min	RPM	%
13	1	700	90	1000	98
8	2	800	90	1100	90.2
9	3	700	80	900	87.5
10	4	700	100	900	87.8
2	5	800	80	1000	88.2
1	6	600	80	1000	82.1
12	7	700	100	1100	89
4	8	800	100	1000	90.5
6	9	800	90	900	85
11	10	700	80	1100	87.4
14	11	700	90	1000	98
7	12	600	90	1100	83.3
3	13	600	100	1000	81.2
5	14	600	90	900	79

Table 2: Composition of the optimized topical gel formulation

Ingredients	Quantity
Water	11 mL
Carbopol	700 mg
Butylated hydroxy toluene	150 mg
Methyl Paraben	150 mg
Polyethylene Glycol	0.24 mL
Glycerol	0.24 mL
Sodium Hydroxide (NaOH)	10.5 mg
Cefepime Hydrochloride (Drug)	2500 mg

Table 3: Evaluation profile of Cefepime HCl gel formulations (F1–F8)

Parameter	Evaluation
Color	Pale yellow
Odor	Pungent
Appearance	Semisolid
Texture	Smooth and homogeneous
Type of smear	Non-greasy
Removal	Easy and quick

Effect of time

As expected in controlled-release formulations, drug release increased with time. All formulations demonstrated time-dependent release profiles, supporting the sustained-release characteristics of the gel [28].

Effect of agitation frequency

Variation in agitation frequency influenced drug release, as increased frequency typically enhances drug diffusion by reducing boundary layer thickness. Higher frequencies promoted faster drug release due to improved molecular mobility within the release medium. These trends are illustrated in Figs. 1-3, which demonstrate the respective influence of Carbopol concentration, time, and frequency on cumulative drug release [29].

pH assessment

The pH values of the gel formulations ranged from 6.5 to 6.8, well within the acceptable range for dermal applications (5.5–7.5), ensuring skin compatibility. Formulation F5 exhibited a pH of 6.8, considered optimal for topical use. Minor pH variations across batches were attributed to differing levels of sodium hydroxide used during Carbopol neutralization [30].

Viscosity

Viscosity values of the formulations ranged from 24.12 Pa·s (F2) to 28.72 Pa·s (F1). The higher viscosity of F1 reflects a denser gel matrix, providing greater film-forming ability and potential for prolonged skin retention. Lower viscosity in F2 may improve spreadability but could compromise retention time. All formulations maintained structural integrity, confirming stability throughout the evaluation period [16].

Spreadability

Spreadability values varied between 6.9 cm (F6) and 8.2 cm (F5). F5 demonstrated the highest spreadability, likely due to an optimal balance between polymer concentration and viscosity, promoting ease of application and uniform drug distribution. In contrast, F6's lower spreadability indicates a more rigid matrix [31].

Extrudability

All formulations exhibited good extrudability characteristics. Formulations F4 and F5 required slightly higher force to extrude, possibly due to increased viscosity or enhanced internal gel strength. Nonetheless, all formulations fell within acceptable performance parameters for patient use [32].

Overall evaluation

Among the formulations, F5 emerged as the most suitable candidate based on its optimal spreadability, favorable viscosity, and balanced ph. These characteristics suggest it would provide an effective and patient-compliant delivery system for topical administration [33].

Drug content uniformity

Three randomly selected gel samples were tested to assess drug content uniformity using UV-Visible spectrophotometry. The drug content varied significantly between samples, ranging from 53.9% to 104.6%. Sample 1 showed a deviation of 10.9%, while Samples 2 and 3 showed deviations of 28.3% and 39.2%, respectively. These results suggest inadequate mixing or non-uniform dispersion during formulation, which must be addressed to ensure dosage consistency and therapeutic reliability [34]. Experiments represent triplicate values.

***In vitro* drug release**

In vitro drug release from the gel was evaluated over 12 h using a Franz diffusion cell. The release profile showed an initial burst (28% at 0.5 h), followed by a sustained release phase, with cumulative drug release reaching 98% at 12 h. This biphasic release pattern is ideal for prolonged therapeutic effect in Table 2 [35].

Antibacterial activity

Antibacterial efficacy of the Cefepime HCl–L-Arginine gel was assessed against *S. aureus* and *P. aeruginosa* using the agar well-diffusion method. *S. aureus* showed increased sensitivity with rising concentrations: 10 mm (125 µg), 11 mm (500 µg), and 12 mm (1000 µg). *P. aeruginosa* exhibited no inhibition at 125–500 µg; however, a 10 mm inhibition zone was observed at 1000 µg. Streptomycin (positive control) demonstrated strong activity: 27 mm for *S. aureus* and 24 mm for *P. aeruginosa*. DMSO (negative control) showed no activity. These results indicate moderate efficacy against Gram-positive bacteria and weak activity against Gram-negative strains. Further optimization, particularly for Gram-negative targets, is recommended [36].

DISCUSSION

DOE analysis

A 3^2 factorial design enabled a systematic evaluation of the effects of three formulation Table 1 variables–Carbopol concentration, agitation frequency, and sampling time–on drug release. This approach reduced the experimental load while allowing insight into interaction effects among variables. As expected, increased Carbopol concentration resulted in decreased drug release due to the formation of a denser gel matrix, which impeded drug diffusion. This behavior aligns with previous studies showing that higher polymer concentrations create tighter Table 1 networks, slowing

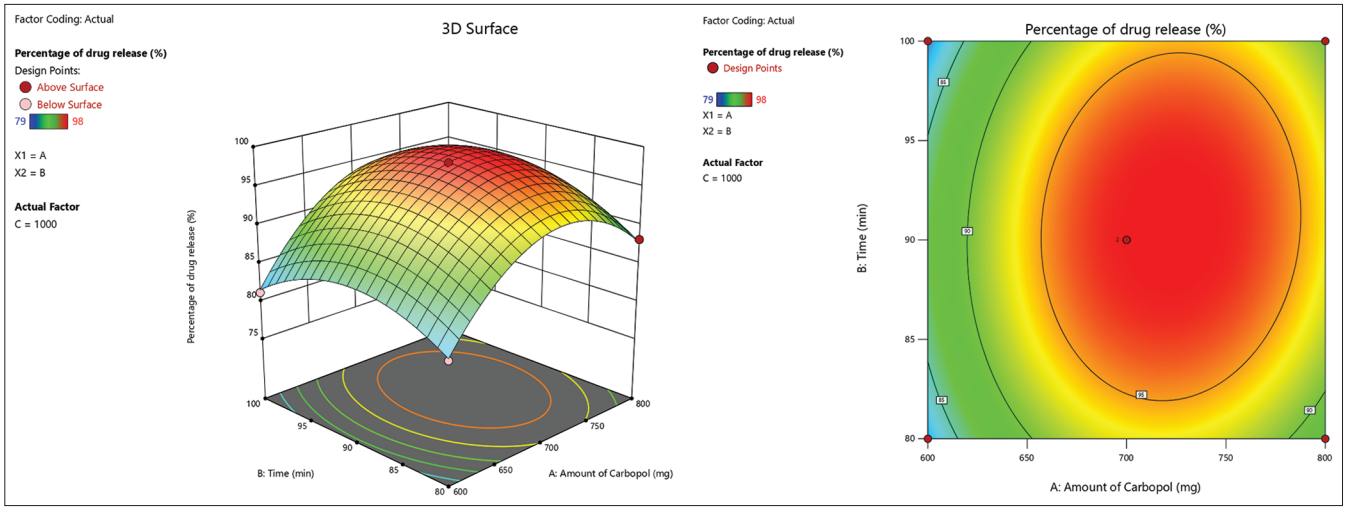

Fig. 1: Effect of amount of Carbopol on percentage of drug release

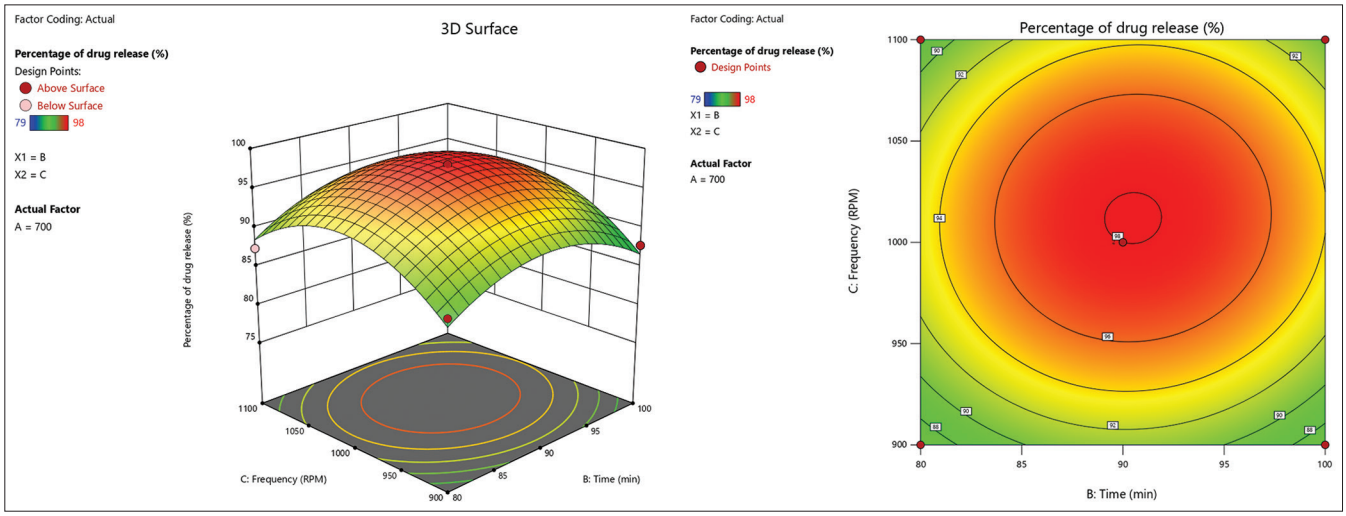

Fig. 2: Effect of time on percentage of drug release

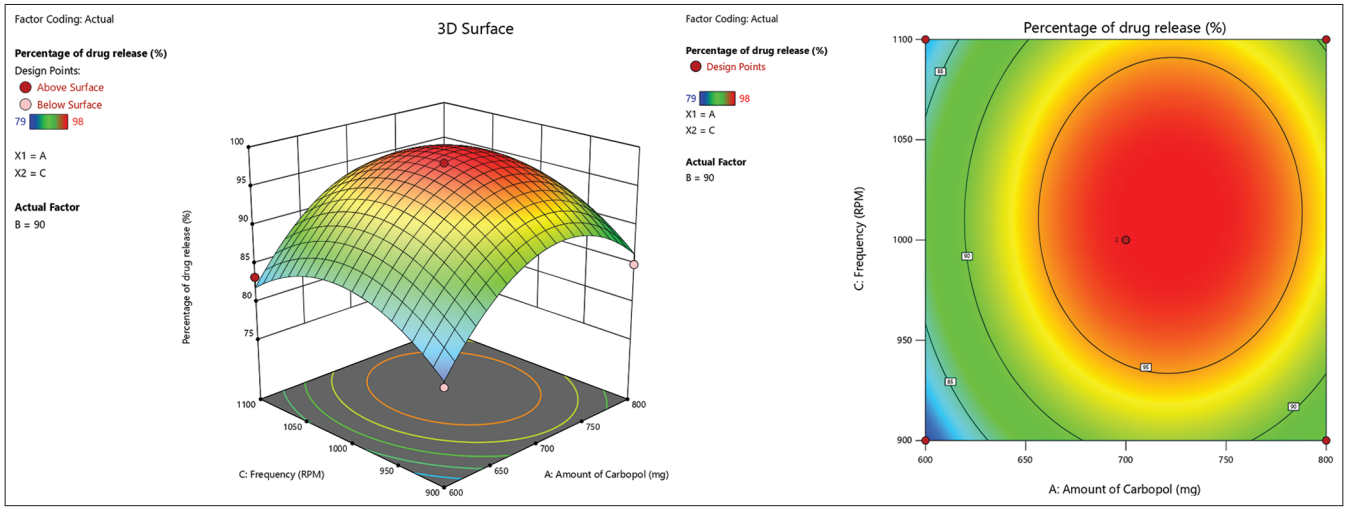

Fig. 3: Effect of frequency on percentage of drug release

both water ingress and drug migration [37]. Drug release was positively correlated with time, demonstrating a sustained-release profile attributable to the structured network formed by Carbopol. Notably, approximately 98% of the drug was released within 12 h, indicating that the formulation may maintain therapeutic levels across a typical dosing interval [26].

Table 4: Evaluation of Cefepime HCl gel formulations (F1–F8)

Formulation	Appearance	Homogeneity	Spreadability	Viscosity	Drug content	pH
F1	Pale yellow	Clear	7±0.07	28.72±0.15	98.73±0.12	6.7±0.02
F2	Pale yellow	Clear	7±0.07	24.12±0.12	97.86±0.28	6.5±0.02
F3	Pale yellow	Clear	8±0.70	27.32±0.15	98.85±0.10	6.5±0.02
F4	Pale yellow	Clear	7±0.70	26.67±0.13	99.03±0.53	6.7±0.04
F5	Pale yellow	Clear	8±0.70	27.06±0.11	99.86±0.40	6.8±0.02
F6	Pale yellow	Clear	6±0.70	26.48±0.13	98.15±0.10	6.8±0.03
F7	Pale yellow	Clear	7±0.70	27.28±0.17	98.8±0.08	6.7±0.02
F8	Pale yellow	Clear	7±0.07	27.16±0.12	98.6±0.08	6.6±0.02

The data represent mean±SD of three experiments (n=3)

Table 5: Drug content uniformity of samples

Sample	Wavelength (nm)	Absorbance (A)	Drug content (%)	% Deviation from mean
Sample-1	273.5	0.670	67.0	10.9
Sample-2	265.5	0.539	53.9	28.3
Sample-3	273.5	1.046	104.6	39.2

Table 6: Cumulative percentage of drug released

Time (h)	Drug release (%)
0.5	28
1	44
2	60
4	78
6	88
8	93
12	98

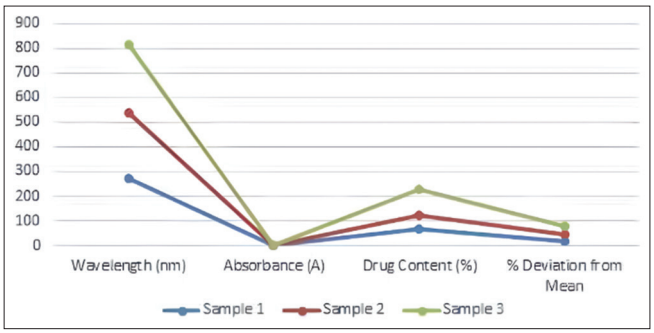

Fig. 4: Comparative analysis of wavelength, absorbance, drug content, and deviation in gel samples

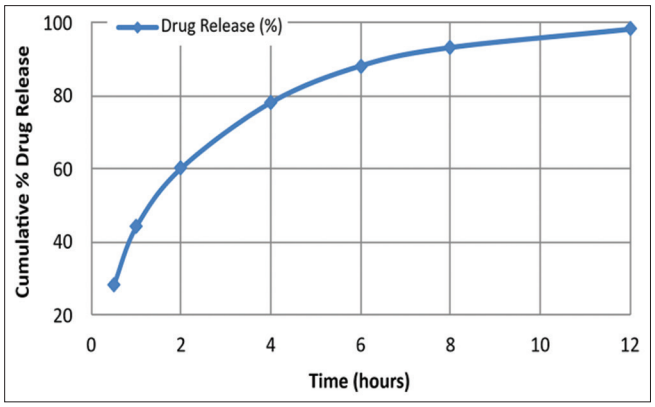

Fig. 5: Cumulative % drug release versus time graph

Physicochemical characteristics

All gel formulations were stable in color and consistency, showing no signs of phase separation. This suggests good physical and chemical compatibility between Cefepime HCl and excipients such as L-Arginine, PEG, Table 3 [38].

PH assessment

The pH values of all formulations (6.5–6.8) remained within the dermally acceptable range (5.5–7.5). Maintaining near-neutral pH is essential not only for skin compatibility but also for preserving the stability of β-lactam antibiotics such as Cefepime HCl, which are prone to hydrolysis in acidic or basic environments [39].

Viscosity and spreadability

Viscosity and spreadability were found to be inversely related. F1, with the highest viscosity (28.72 Pa·s), exhibited slightly reduced spreadability, whereas F5 provided an optimal balance, offering adequate film retention and ease of application. For example, F7 (viscosity = 27.29 Pa·s) exhibited spreadability of 7.2 cm, which was less than F5 (8.2 cm), highlighting how viscosity influences user experience. Ideal formulations must maintain structural integrity while being easy to apply Optimization of Potential Nanoemulgels for Boosting Transdermal Glimepiride Delivery and Upgrading Its Anti-Diabetic Activity [40].

Extrudability

All formulations showed acceptable extrudability. F4 and F5 required marginally greater pressure, likely due to higher viscosity or more cohesive gel structures. Nevertheless, their extrusion performance remained within practical limits. These findings are important for user compliance and packaging considerations [41].

Drug content uniformity

Drug content uniformity ranged from 53.9% to 104.6%, indicating a significant deviation that may affect dosage accuracy. This variation likely results from inadequate mixing or drug dispersion during formulation. Future optimization could involve high-shear mixing or pre-solubilizing the drug in a co-solvent to enhance uniformity [42].

***In vitro* drug release**

The formulation displayed a biphasic release profile: an initial burst followed by sustained diffusion. This release pattern benefits topical therapy by providing rapid onset and prolonged antimicrobial activity. The presence of PEG and glycerol likely improved hydration, aiding drug mobility through the gel matrix Figs. 4 and 5 [43].

Antibacterial activity

The antibacterial efficacy of the gel was higher against *S. aureus* (Gram positive) compared to *P. aeruginosa* (Gram negative). The structural differences between these organisms likely account for this discrepancy. Gram-positive bacteria lack the outer membrane found in Gram negatives, allowing easier penetration of hydrophilic drugs such as Cefepime HCl [44]. The formulation showed only weak inhibition against *P. aeruginosa* at high concentrations (1000 µg), while *S. aureus* showed moderate sensitivity even at lower doses. Streptomycin, the positive control, showed significantly larger inhibition zones, highlighting the need for formulation enhancement. Although the

Table 7: Antibacterial activity (Agar well-diffusion method)

Sample	Zone of inhibition in mm											
	Staphylococcus aureus						*Pseudomonas aeruginosa*					
	R1	R2	R3	Mean	SD	Mean±SD	R1	R2	R3	Mean	SD	Mean±SD
F4	12	12	13	12.33	0.4714	12.3±0.4	9	9	10	9.33	0.4714	9.3±0.4
F5	14	14	15	14.33	0.4714	14.3±0.4	12	12	13	12.33	0.4714	12.3±0.4

The data represent mean±SD of three experiments (n=3)

presence of L-Arginine may have contributed to antimicrobial potential via nitric oxide pathways, the effect was not sufficient to significantly enhance efficacy against resistant strains. Further studies could explore synergistic combinations with other permeation enhancers or antimicrobials [45-47].

Optimized formulation and statistical findings

Formulation F5 was identified as the most promising candidate, demonstrating optimal pH, viscosity, spreadability, extrudability, and release kinetics. *In vitro* testing confirmed its capability to release 98% of Cefepime HCl over 12 h. While moderate antibacterial activity was observed, especially against *S. aureus*, improvements are necessary to enhance activity against Gram-negative bacteria. One-way ANOVA confirmed that the independent variables (Carbopol amount, time, and agitation frequency) significantly influenced drug release ($p<0.05$). The formulation was further optimized using Design-Expert® software (Version 13) with RSM, providing an evidence-based path toward formulation refinement [48]. This study demonstrates the feasibility of developing a topical antibacterial gel incorporating Cefepime HCl and L-Arginine with controlled-release properties. While F5 stands out as the most effective formulation among those tested, further work is necessary to improve drug content uniformity and broaden antibacterial coverage–particularly against Gram-negative pathogens [49].

CONCLUSION

An optimized antibacterial gel containing Cefepime HCl and L-Arginine was successfully developed using a Box–Behnken design. The study systematically evaluated the influence of Carbopol concentration, mixing time, and agitation frequency on the formulation's drug release profile, physicochemical properties, and antibacterial efficacy. Among the tested formulations, F5 demonstrated the most favourable characteristics, including an appropriate pH, excellent spreadability, suitable viscosity, and good extrudability—making it ideal for topical application. The formulation exhibited a sustained drug release over 12 h, with a cumulative release of approximately 98%, indicating strong extended-release performance. Antibacterial studies revealed moderate activity against *S. aureus* and limited efficacy against *P. aeruginosa*, particularly at higher concentrations. The statistical analysis confirmed that the selected independent variables significantly influenced drug release ($p<0.05$). The optimization process, carried out using Design-Expert® software (Version 13) and RSM, identified the ideal formulation parameters.

In conclusion, the optimized gel formulation shows promise as a topical treatment for Gram-positive bacterial infections. However, future research should focus on enhancing drug content uniformity and antibacterial efficacy, particularly against resistant Gram-negative organisms, to broaden its therapeutic application.

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AUTHOR CONTRIBUTION

Sandhiya Sivakumar designed the formulation using Design of Experiments (DOE) and analytical characterisation. Structured and edited the manuscript. Mohamed Kaif Haja Maideen designed the formulation using Design of Experiments (DOE) and analytical characterisation. Structured and edited the manuscript. Akash Steppi designed and executed the formulation using Design of Experiments (DOE) and analytical characterisation. Structured and edited the manuscript. Dr Renukadevi Jeyavelkumaran provided conceptualization, overall supervision of the research work, ensured methodological validation, critically revised the manuscript, and approved the final version for submission. All authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this study. The research and its outcomes were not influenced by any personal or financial relationships.

ETHICAL APPROVAL

Not applicable. This study does not involve any animal or human participants requiring ethical approval.

CONSENT STATEMENT

Not applicable. No human participants, personal data, or identifiable images were used in this study.

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