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Original Article

Optimization of Novel Topical Cubosomal Gel Containing Lidocaine Hydrochloride by Design of Experiment

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ABSTRACT

Background: Cubosomal gels have emerged as a favourable mode for delivery of drugs, contributing to release of drug in controlled mode with increased effectiveness. The study aimed to develop an optimized cubosomal gel formulation containing Lidocaine Hydrochloride for sustained pain relief, particularly in post-operative pains. **Materials and Methods:** Bottom-up method of approach was employed to prepare cubosomes utilizing Glyceryl Mono Oleate (GMO) and Poloxamer 407 (P-407). The formulations underwent comprehensive characterization analysis, entrapment efficiency determination and zeta potential measurement. Utilizing Design of Experiment methodology, an optimized formulation LH8, prepared with medium concentration of GMO and high concentration of P-407 was derived. Subsequently, LH8 was incorporated into a gel base and evaluated for various parameters including viscosity, drug content, extrudability, and spreadability. *In vitro* and *ex vivo* permeation studies were conducted to assess the sustained release profile. **Results:** The optimized formula LHG8 prepared with medium concentration of Carbopol and high concentration of Propylene glycol showed flux of 38.29 µg/cm^2, 66.8% permeability, 7.1 cm spreadability and 91.2% extrudability. Importantly, LHG8 demonstrated excellent sustained release activity, indicating its potential for effective post-operative pain management. **Conclusion:** This study highlights the promise of cubosomal gels as a viable strategy for optimizing drug delivery, with implications for improving patient outcomes in pain management

Keywords: Cubosomal Gel, Drug Delivery, Factorial Design, Lidocaine Hydrochloride, Optimization, Sustained Release.

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INTRODUCTION

Cubosomes, with their enormous surface area, low viscosity, and stability over dilution levels, present a viable option for nano-sized topical administration systems.[1,2] These lipid vesicles are perfect for transporting hydrophilic, lipophilic, and amphiphilic medications because they resemble biological membranes.[3] Cubosomes are flexible that can be injected intravenously, subcutaneously, or orally.[4] They work well with gel systems such as Carbopol[5] and Hydroxy Propyl Methyl Cellulose. The objective of this research was to develop a Lidocaine Cubosomal topical gel to maximize its therapeutic efficiency and minimize any gastrointestinal adverse effects,[6] especially those associated with gastrointestinal irritation.[7,8]

MATERIALS AND METHODS

Materials

Poloxamer 407 was generously provided as a sample gift by Daewoong Pharmaceuticals in Hyderabad. Mohini Organics Pvt. Ltd, Mumbai supplied Glyceryl Monooleate as a gift sample. Additionally, Genuine Chemical Co. in Mumbai contributed Carbopol 940 as gift sample. Analytical quality grades of Triethanolamine, Propylene glycol, Glycerine, and Methyl paraben were used in the current work.

Methods

Preparation and Characterization of Lidocaine hydrochloride cubosomes

To optimize Lidocaine HCl-loaded cubosomes, a 3^2 factorial design experiment was conducted, resulting in 9 trials repeated three times each (LH1-LH9). Using Design Of Experiment® software, statistically validated equations were derived to develop an optimized batch.[9] The formulation involved varying concentrations of lipid (GMO) and stabilizer (P-407), followed by cubosome formation through low-energy homogenization and vortexing.[10] Characterization included visual inspection

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for dispersion quality, Scanning Electron Microscopy and Transmission Electron Microscopy for structural analysis, particle size analysis, zeta potential determination, and entrapment efficiency evaluation.[11] *In vitro* drug release and *ex vivo* permeability studies assessed the release kinetics and skin permeation of Lidocaine HCl from the cubosomal dispersion. The assessments were conducted, documented and published.[12]

Preparation of Lidocaine hydrochloride loaded cubosomal gel

Gel formulations were systematically designed employing a 3^2 factorial design, wherein Carbopol and Propylene glycol levels were varied across nine trials. Each trial encompassed permutations of low, medium, and high levels for both factors facilitating a comprehensive exploration of formulation parameters.[13] Cubosomal topical gels were prepared via dispersion method.[14] Gelling agents underwent a 4-hr hydration period in water. Propylene glycol was then introduced into the gel with consistent mixing. Triethanolamine followed to adjust the gel's pH, while glycerol balanced its viscosity.[15] The optimized cubosomal dispersion was accurately incorporated and mixed. Methyl paraben served as a preservative. The resulting gels were carefully packaged into glass containers and stored in a refrigerator for optimal preservation.[16]

Characterization of a novel gel formulation containing Lidocaine hydrochloride

Fourier transform infrared spectroscopy analysis

The formulated gel, comprising drug, gelling agents, physical mixture of all components of cubosomal dispersion undergoes FTIR spectroscopy across the 4000 to 400 cm^{-1} range to interpret any potential chemical interactions or incompatibilities.[17]

pH measurement

The pH of the gel is determined after calibrating the digital pH meter. The electrode was submerged in the cubosomal gel sample until the reading is stabilized, at which point the pH value is recorded, ensuring consistency and appropriateness for intended applications.[18]

Viscosity determination

The viscosity of the gel formulations is accurately quantified, providing crucial insights into its rheological properties and applicability using Brookfield viscometer equipped with a T-Bar spindle (T64).[19] After maintaining viscosity throughout the process, the helipath T-bar spindle was moved up and down, resulting in viscosity at several points along the path due to the factors like temperature, pressure, and sample size, among others.

Drug content Analysis

The concentration of the therapeutic compound within the gel is determined by dissolving 1 gof cubosomal gel in 100 mL phosphate buffer solution. After filtering the stock solution, appropriate dilutions were made to create aliquots of various concentrations, which were then examined with subsequent measurement of absorbance at 230 nm using a UV spectrophotometer.[20]

Extrudability

The extrudability procedure for a gel typically involves loading a predetermined amount of gel into a collapsible tube. Gradually, force or weight is applied to the tube to expel the gel and the percentage of gel dispensed is calculated.[21]

Spreadability

Spreadability calculation of the gel involved carefully spreading 1 g of the formulation onto a glass slide with a 2 cm diameter. A known amount of mass was placed on the lower slide followed by placing another slide over it. Subsequently, the diameter of the spread gel circle was accurately measured. Spreadability was then calculated as the difference between the Final Diameter (d2) and the Initial Diameter (d1).[22]

Optimization and validation

Utilizing Stat-Ease Design expert software, and optimization strategy that made use of the desirability function, an optimized cubosome formulation that meets the prerequisites laid out earlier for EE, particulate size, Poly Dispersibility Index and Zeta Potetial was being emerged out. The factors were assessed, and the ideal formula was proposed. Every dependent variable was analyzed to check whether it fits inside the 95% PI or not. Additionally, the actual and anticipated values were contrasted using the percentage prediction error.

Drug permeation: *In vitro* study

Permeation studies of drug were conducted by *in vitro* means utilizing a Franz diffusion cell maintained at standard temperature conditions. A pH 7.2 Phosphate Buffer served as the receptor medium. Samples were taken out at predetermined times (1, 2, 3, 4, 5, 6, 12, and 24 hr), replaced with new buffer, and then examined at 230 nm with UV spectrophotometer to calculate the quantity of medication that has permeated.[23]

Drug permeation: *Ex vivo* study

Utilizing excised animal skin mounted in a Franz diffusion cell, the permeation behavior of the gel formulation is further elucidated. Samples were collected at periodically (1, 2, 3, 4, 5, 6, 12, 24 hr) and examined with a UV-visible spectrophotometer at 230 nm. The receptor phase was promptly replaced by an equivalent volume of new buffer solution.[24]

Skin irritation studies

To ensure safety of the skin on application of cubosomal gel, skin irritation tests were conducted on male wistar rats.[25] The test was performed following the OECD Guidelines test no. 404 "Acute

dermal irritation/corrosion" in conducting skin irritation studies on rats following the Institutional Animal Ethical Committee (IAEC) approval as per official guidelines with study protocol number 04/IAEC/VCOP/2022.

In vivo Studies

Wistar rats (male) were selected to conduct *In vivo* studies. Rats were categorized up into the following treatment groups: Normal saline was given to Group I, while oral lidocaine solution was given to Group II; marketed Lidocaine gel was administered to Group III; Optimized Lidocaine Hydrochloride cubosomal gel was administered to Group IV. Following treatment, at various intervals, blood was extracted from the retroorbital sinus viz. 1, 2, 4, 6, 12, 24 hr. The samples were centrifuged at a speed of 10000 rpm and for duration of 10 min at 4°C to isolate the clear plasma. The amount of drug present in plasma was estimated using HPLC.[26] The mobile phase was 2% formic acid in acetonitrile-water (70:30). The flow rate was fixed at 1 mL/min. Bupivacaine was used as an IS. All calculations were based on peak-area ratios of lidocaine and Internal standard. Stock solution of (1000 µg/mL) was prepared by accurately weighing 10 mg of LDH in 10 mL volumetric flask. The drug was dissolved in methanol and the solution was diluted to volume. Further dilutions were made from this stock solution and the injection volume was kept 20 µL. A calibration curve was plotted between concentrations against their respective area for LDH.[27]

Stability Studies

Accelerated stability tests were performed on the prepared cubosomal gels as per ICH guidelines.[28] Samples were withdrawn and evaluated for various rheological parameters.

RESULTS

Studies on drug-excipient compatibility were conducted utilizing FTIR. Each element's FT-IR spectra as well as their physical combination were analyzed to identify any interactions which would impact functionality and, in turn, stability as well as compatibility. The data obtained from the spectra of pure drug and Lidocaine cubosomal gel is depicted in Table 1.

There was not much deviation observed in the peak stretchings of Aromatic C=C, >C=O, C-H, C-N, N-H bonds of pure drug and the formulation of cubosmal gel which clearly established evident drug- excipient compatibility.

Lidocaine hydrochloride loaded cubosomal gel

The study utilized a 3^2 factorial design to investigate the impact of two independent variables, Carbopol (X1), along with propylene glycol (X2), adjusted at three different levels. This design yielded nine trial formulations, each representing a combination of high, middle, and low levels of the variables, as illustrated in Table 2. The objective was to analyze how these independent factors influence the response parameters of flux, permeability (%), Spreadability and extrudability (%).

The gel's pH, measured at 6.9±0.32, falls within the skin's acceptable pH range. Better drug content, pH, viscosity, spreadability, extrudability, and *in vitro* and *ex vivo* penetration were all demonstrated by the cubosomal gel formulation. Optimized formula LHG8 showed flux of 38.29 µg/cm^2, 66.8% permeability, 7.1 cm spreadability and 91.2% extrudability. 1 g of the cubosomal gel was dissolved in 10 mL to assess the amount of lidocaine present, with subsequent spectrophotometric measurement at λ_{max} 230 nm, revealing a drug content of 98%. The software generates response surface plots, contour plots as shown in Figure 1.

Contour plots complement the interactions between Carbopol and PG on the the flux and characterization parameters of the prepared gels (LH1-LH9). The contour plots exhibits that transdermal flux value increased as the concentration of Carbopol was increased. This was normal on the grounds that the interlinkage between Carbopol and PG would prompt to enhance of flux value and other characterization parameters of the gel. Table 3 shows that all models were significant (p<0.05) for all response parameters analysed.

In vitro permeation studies

The optimized batch was the focus of the *in vitro* permeation study, which was carried out in a pH 7.2 buffer. The cumulative amount of drug permeated increased to 82.92% after a 24-hr period (Figure 2).

Ex vivo studies

The skin permeation profile of optimized cubosomal gel formulation (LHG8) showed similar permeation pattern as that of the *In vitro* release profile across the gelatin membrane with a drug permeation rate of 64.27±0.89% at the end of 24 hr.

In vivo studies

Figure 3 depicts the plasma concentration-time profiles of fasting rats given single doses of oral Lidocaine solution, lidocaine-marketed gel, and topical lidocaine-loaded cubosomal gels. The individual lidocaine plasma concentration-time curves were used to calculate all the possible bioavailability parameters. The mean values±SD are shown. The relative bioavailability of transdermal cubosomal dispersions increased significantly (p<0.05), due to their skin-like structure, allowing for flexible delivery of hydrophilic and lipophilic medicines. The other factor, GMO has altered the stratum corneum's lipid bilayers' intercellular ordered structure and made them more fluid. Additionally, the drug can penetrate deeper into the stratum corneum due to Poloxamer's presence in cubosomes, which changes the lipid arrangement, increases fluidity, and eventually improves transdermal drug penetration.

Table 1: **IR Spectra data of pure drug and Lidocaine Hydrochloride cubosomal gel formulation.**

IR Spectra	Peak functional groups (Stretching; cm^{-1})				
	C=C	>C=O	C-H	C-N	N-H
Pure drug	2191	1654	2976	1297	3387
Optimized Lidocaine Cubosomal gel formulation (LHG8)	2214	1650	2924	1280	3383

Table 2: **Formulation of gel base for preparation of Lidocaine HCl cubosomal gel and various independent and dependent factors, levels with their values.**

Ingredients	LGH1	LHG2	LHG3	LHG4	LHG5	LHG6	LHG7	LHG8	LHG9
Optimized LHDC dispersion (equivalent to (%w/w)	2	2	2	2	2	2	2	2	2
Carbopol 940 (% w/w)	1	1	1	1.5	1.5	1.5	2	2	2
Propyleneglycol (% w/w)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Triethanolamine (%w/w)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Glycerine (%)	1	1	1	1	1	1	1	1	1
Methyl paraben (%w/w)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Water	(upto 100% w/w)								

Note: Independent Components=Carbopol %w/w, Propylene glycol %w/w, High Level= (+1), Middle level= (0), Low level=(-1), Dependent components=Flux (µg/cm^2), Permeability (%), Spreadability(cm), Extrudability (%).

The relative bioavailability of transdermal cubosomal dispersions increased significantly ($p<0.05$), due to their skin-like structure, allowing for flexible delivery of hydrophilic and lipophilic medicines. The other factor, GMO has altered the stratum corneum's lipid bilayers' intercellular ordered structure and made them more fluid. Additionally, the drug can penetrate deeper into the stratum corneum due to Poloxamer's presence in cubosomes, which changes the lipid arrangement, increases fluidity, and eventually improves transdermal drug penetration.

Skin irritation studies

After completing the skin irritation test, it was inferred that Cubosomal gel was safe and non-irritating. There was no evidence of erythema or edema (Table 4).

Stability studies

Stability study was conducted over a three-month period under storage conditions of 25°C/60% RH. No major changes occurred in the flux, permeability, spreadability and extrudability with results of the corresponding parameters as 38.29 µg/cm^2, 66.8±0.7%, 7.1±0.97 cm and 91.2±0.75% respectively when compared to those obtained after the end of study period noted as 38.12 µg/cm^2, 66.8%±0.61, 7.1±0. 83 cm and 91.2±0.68% respectively.

DISCUSSION

Lidocaine-filled cubosomes were produced using the Bottom-Up technique led by Quality by Design (QbD) principles. Formulation procedures were chosen based on a comprehensive literature review. Optimization data was analyzed using Design-Expert® software to establish polynomial equations. DOE software evaluated formulation batches for various parameters. Using the homogenization technique, drug entrapped cubosomes were produced. According to the data from the spectral diagrams, lidocaine did not have any interactions with any other components, either singly or in combination, with respect to its peak.

To analyze the data effectively, Design-Expert software was employed to generate polynomial model equations. These equations encompassed both the individual major factors and interaction factors, enabling a comprehensive understanding of the relationships between the variables and the response parameters.

$$Y1=+31.65+11.85 * X1+3.05 * X2-2.20 * X1*X2 -5.90 * X12-0.43 * X22$$

$$Y2=+54.77+11.80 * X1-2.45 * X2-0.78 * X1*X2 -2.45 * X12+2.60 * X22$$

Y3=+6.21+0.85 * X1+0.19 * X2-0.46 * X1*X2 -0.12 *

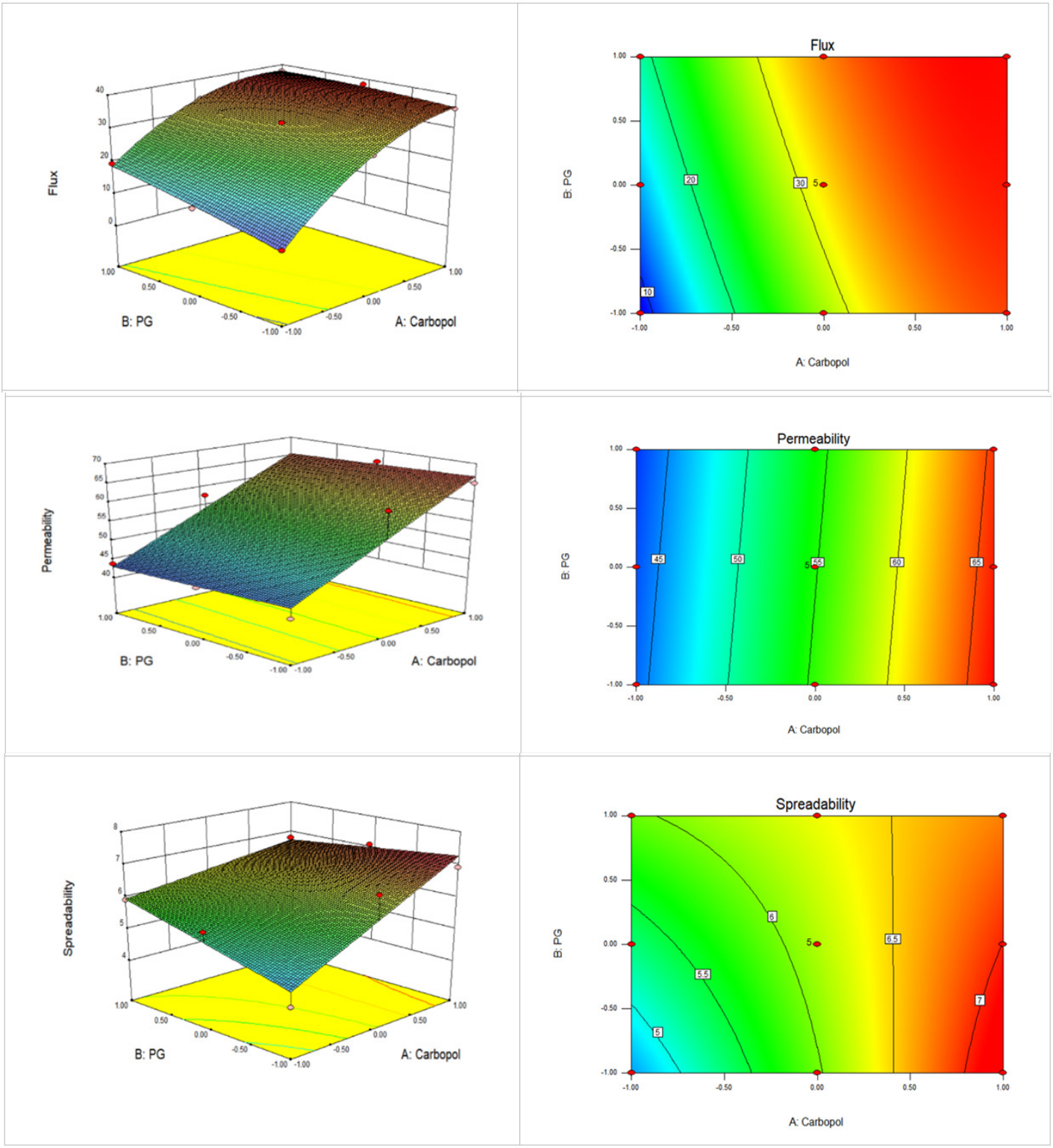
X12+4.48 * X22

Y4=+87.92+6.35 * X1+0.55 * X2-1.40 * X1*X2 -3.22 *

X12-0.72 * X22

Where Y_1, Y_2, Y_3 and Y_4 are the predicted responses.The coded values for the test variables P-407 and GMO are denoted by X_1 and X_2. Following the data input for each of the four responses, two different kinds of models were produced by the software "Design-Expert®".

The terms "X*Y" stand for the combination of these two elements. When the value of a model term is less than 0.05, it is



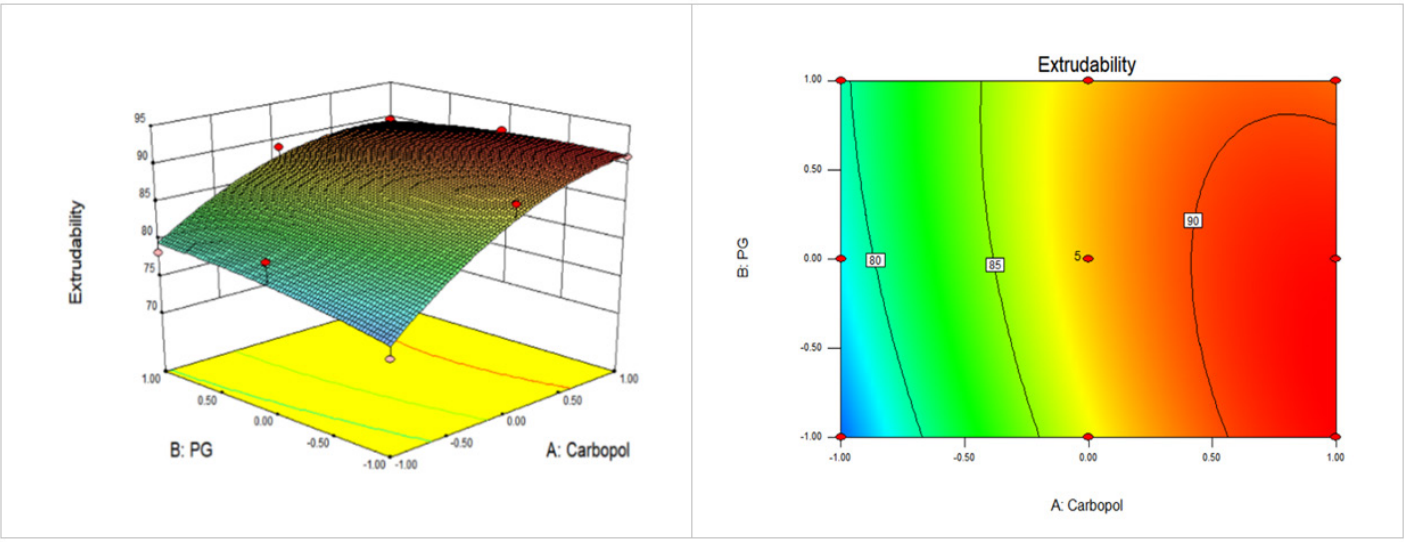


Figure 1: Contour and response surface plots for Flux, Permeability, Spreadability and Extrudability.

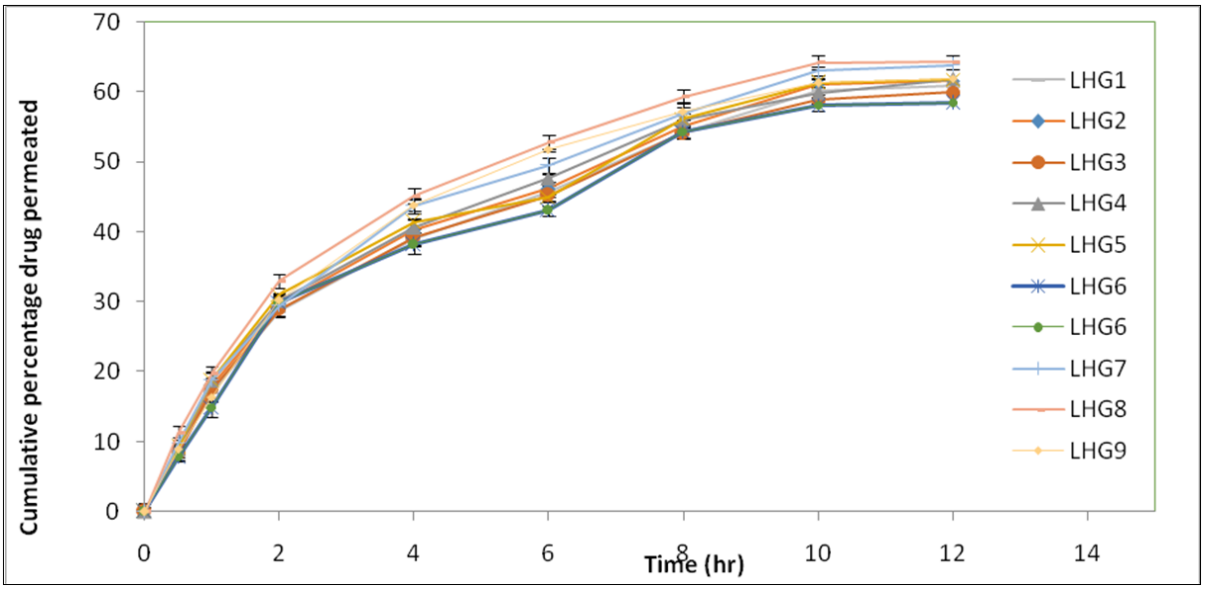


Figure 2: Cumulative amount of drug permeated from Gel formulations of Lidocaine HCl. Each value represents mean±s.d. (*n*=3).

considered significant; when it is larger than 0.05, it is considered insignificant. The outcomes showed a significant reduction in particle size and an improvement in entrapment efficiency. The results demonstrated that the generated design space can lower the likelihood of an error.

ANOVA for interaction between PG and Carbopol on extrudability confirmed the existence of Quadratic Model. The Model F-value of the four responses implies the model is significant. There is only a 0.05% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, A++2+- are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The obtained ratios of all four responses as shown in Table 3 indicates an adequate signal. This model can be used to navigate the design space.

In vitro drug release studies were conducted using Franz diffusion cell, revealing that the cubosomal gel exhibited significantly enhanced drug release. The network-like structure that allowed for continuous release may have been facilitated by the combined effect of P-407 in cubosomal dispersion and the carbopol contained in the gel formulation.

From the plasma concentration curve, it was elucidated that the oral dosage of Lidocaine resulted in a C_{max} of 8.13±0.67 µg/mL at T_{max} 1 hr, indicating fast absorption. However, significantly a greater C_{max} value (11.31 µg/mL at 4 hr) was noted following transdermal cubosomal gel administration. The substantially delayed higher C_{max} values of lidocaine cubogel indicate that the drug is slowly released from the cubosomal dispersion. The produced cubogel's

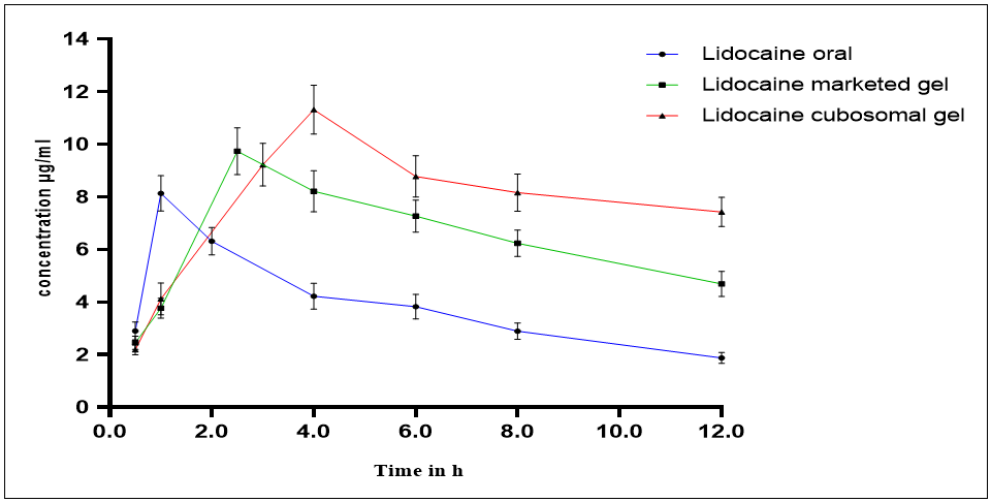


Figure 3: *In vivo* % drug permeated of Optimized formulation. Each value represents mean±s.d. (*n*=3).

Table 3: Regression analysis responses, along with the remarks generated by DoE software.

Model	R^2	Adjusted R^2	Predicted R^2	SD	%CV	*p*-value Prob>F
Y1	0.9972	0.9953	0.9727	0.64	2.23	0.0001
Y2	0.9372	0.8924	0.5281	2.74	5.00	0.0004
Y3	0.8511	0.7447	0.3028	0.37	6.00	0.0082
Y4	0.9350	0.8886	0.4888	1.70	1.98	0.0005

Table 4: Skin irritation studies of optimized formulations.

F.code	60 min		24 hr		48 hr	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
LHG4	0	0	1	0	1	0
LHG7	0	0	0	0	1	0
LHG8	0	0	0	0	0	0
LHG9	0	0	1	0	0	0

Each value represents mean±s.d. (*n*=3). Note: 0= No sign of erythema and edema, 1= Very sign of slight erythema and edema, 2= Well defined sign of erythema and edema, 3= Moderate to severe sign of erythema and edema, 4= Severe sign of erythema and edema.

AUC_{0-12} was 212.75 µg.hr/mL, whereas the oral solution's was 147.64 µg.h/mL According to AUC_{0-12}, lidocaine cubosomal gel have a relative bioavailability 1.44 times greater than that of oral solution. The relative bioavailability of transdermal cubosomal dispersions increased significantly ($p<0.05$), due to their skin-like structure, allowing for flexible delivery of hydrophilic and lipophilic medicines. The other factor, GMO has altered the stratum corneum's lipid bilayers' intercellular ordered structure and made them more fluid. Additionally, the drug can penetrate deeper into the stratum corneum due to Poloxamer's presence in cubosomes, which changes the lipid arrangement, increases fluidity, and eventually improves transdermal drug penetration.

The final optimized gel formulation was assessed for discoloration and phase separation. Visual inspection of the optimized formulation was conducted over a three-month period under storage conditions of 25°C/60% RH. Neither indications of phase seggregation nor precipitation of drug were detected in the optimized formulation after stability studies. Subsequent storage of latterly formulated Lidocaine encapsulated cubogel, the permeability, flux, spreadability and extrudability of the gels were compared with results attained prior storage, demonstrating the stable behavior of gel when preserved at 25°C±2°C.

CONCLUSION

Topical cubogel was developed for overcoming the side effects of oral administration of the drug. A factorial 3^2 design was used successfully to formulate Lidocaine hydrochloride Cubosomal Gel. The optimized formulation LHG8 depicted appreciable evaluation parameters and could further be utilized to investigate *in vivo* studies elaborately to establish its safety and effectiveness. Further, future studies of this research would include scale up

studies of the optimized gel formulation along with a detailed preclinical safety, toxicity and efficacy studies.

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ABBREVIATIONS

LDH: Lidocaine Hydrochloride; **LDH:** Lidocaine Hydrochloride; **PB:** Phosphate buffer; **FTIR:** Fourier transform infrared; **GMO:** Glyceryl mono oleate; **P407:** Poloxamer 407; **DoE:** Design of Experiment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

In vivo studies of prepared Lidocaine cubosomal gel were conducted with the approval of Institutional Animal Ethical Committee (IAEC) with study protocol number 04/IAEC/VCOP/2022.

SUMMARY

The current study effectively developed Lidocaine Hydrochloride cubosomal gel employing design of experiment. Lidocaine loaded cubosomal dispersions were prepared using Bottom up method. Optimized cubosomes were incorporated into gel base using dispersion method. Nine formulations were developed, which were subjected to various characterization studies including spreadability, extrudability, viscosity, *In vitro* and stability studies. *In vivo* studies were carried out for optimized cubosomal gel formulation (LHG8) with IAEC approval. Further studies can be carried out for its effectiveness and extensive approach.

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