ORIGINAL ARTICLE

Investigating the Role of Experimental Variables in Characterization of Lidocaine Hydrochloride Cubosomes by QbD

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Abstract

Aim: The objective of this research work was to analyze the effect of experimental components (homogenization time, speed of homogenization, and time of sonication) on the characterization of cubosomes loaded with Lidocaine Hydrochloride (LH). **Materials and Methods:** The Top-down method was adopted to prepare LH cubosomes using lipid (Glyceryl Monooleate), stabilizer (Poloxamer-407) along with water in varied proportions. 3^2 factorial design was adopted to explain how the two independent factors, that is, homogenization speed and sonication time affect the four responses at varied levels of the processing parameters for the preparation of drug-encapsulated cubosomes. **Results and DiscussiJon:** The most acceptable entrapment capacity was found at a homogenization speed of 50 rpm and sonication time employed for 10 min for the formulation LT8 within the confidence intervals adopted by the 3^2 factorial design. LT8 was found to have Entrapment efficiency (EE) of 75 percent, Zeta potential of 21.5 mV, vesicle size of 150.3± 0.77 nm, and PolyDispersibility Index of 0.278. As Hominization speed increased there was a noticeable improvement in the characterization of cubosomes, however, enhanced characterization parameters were observed in dispersions prepared with a medium level of sonication time. **Conclusion:** A steady dispersion of LH was formed with a high level of speed of homogenization and a medium level of sonication time to attain the desired parameters for the characterization of cubosomes. Therefore, it is concluded that the speed of homogenization of 50 rpm and sonication time of 10 min resulted in a stable dispersion.

Key words: Cubosomes, glyceryl monooleate, lidocaine HCl, poloxamer-407, top-down, variables

INTRODUCTION

There are numerous methods of drug administration, including oral, transdermal, parenteral, and mucosal administration, all of which are dependent on the method of delivery.[1] Among them, the transdermal mode of drug delivery has gained importance as an alternative to traditional drug delivery administered by conventional oral route.[2] Numerous advances in novel transdermal drug delivery have been made, with the primary goal of extending drug release and its duration of action.[3] To effectively administer medications, new transdermal systems, such as cubosomes, nanoparticles, liposomes, transferosomes, and hexosomes have promisingly gained importance as carriers of drugs.[4] By stacking drugs into these vesicular molecules, controlled drug conveyance, a more drawn-out term of activity, and the evasion of continuous medication are improved.[5]

Cubosomes are one of the vesicular methods that are most lauded for their capacity to tune the layer bend of nanoparticle breadth freely across vast scales of length.[6] Their internal structure is made up of a single membrane bilayer which fabricates a network resembling lattice structure utilizing two distinct channels for water, that intertwine but are not connected.[7] These are biocompatible drug transporters and

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are nanostructured, translucent fluid particles made of specific, proportional amphiphilic lipids.[8] These are self-assembling liquid crystalline particles carrying a distinct portion of surfactant to water.[9] These vesicles can self-assemble and can serve as an active drug delivery system.[10] High-energy shatter results in the formation of cubic nanoparticles, which are isotropic resulting in the formation of colloidal attributes with thermodynamic stability.[11]

Lidocaine Hydrochloride (LH), a generally perceived anesthetic drug acting locally, is regularly utilized for overseeing post-surgical torment and giving suggestive help in different circumstances, such as muscle distress, hemorrhoids, and neuralgia.[12] Across this study, cubosomes were figured out to upgrade the remedial viability of Lidocaine while limiting unfavorable impacts, especially those connected with gastrointestinal disturbances.

The goal of this research is to optimize the influence of speed of homogenization and duration of sonication on the characterization of cubosomes by incorporating the drug into a vesicular system for extended and targeted drug delivery. The present work is centered around the arrangement of LH cubosomal scattering. Through cubosomal drug conveyance, controlled delivery of Lidocaine HCl can be accomplished which will be helpful in effective treatment. This sort of medication conveyance can likewise forestall continued dosing of drug.

MATERIALS AND METHODS

Pure drug (Lidocaine HCl) was bought from Yarrow Chemicals. Poloxamer-407 was acquired as a liberal gift test from Daewoong Drugs, Hyderabad. Glyceryl monooleate (GMO), triethanolamine, and methylparaben were bought from Finar Synthetic Compounds, Mumbai.

Pre-formulation studies

To define the drug in accordance with pharmacopeia, pre-formulation studies were carried out. According to compendia specifications, FT-IR spectrophotometer is used to identify the drug by comparing their characteristic peaks[13,14] and for the examination of Lidocaine- Polymer interactions. The similarity investigation of the unadulterated drug, excipients, and drug-excipients combination, that is, Lidocaine HCl, GMO, P-407, and drug-loaded cubosomes was performed. The evaluations are led, reported, and published.[15]

Calibration curve of lidocaine HCl in phosphate buffer

Lidocaine HCl (100 mg) was solvated in pH 7.4 PB in a 100 mL volumetric flask likewise, the final volume was filled to 100 mL with PB to attain a concentrated solution of 1 mg/mL. Successive dilutions were prepared and their respective absorbances were estimated by UV-visible spectrophotometer at λmax 230 nm and the calibration curve was constructed.[16,17]

Preparation of lidocaine HCl loaded cubosomes using the top-down method

Preparation of cubosomal dispersion entrapped with Lidocaine HCl was planned to utilize a 32 factorial model to determine the influence of the duration of sonication and the speed of homogenization that affect the characterization of formed cubosomes. The Top-down approach was opted for formulating dispersions.[18-20] Impact of homogenization speed and sonication time incorporated at stages of minimum, medium, and maximum significant levels was developed using DoE software[21] to determine the impact of the two independent factors on the four dependent elements of characterization of cubosomes, that is, particle size, entrapment efficiency, zeta potential and polydispersibility index (PDI) [Table 1].

Characterization of drug-loaded cubosomes

Visual inspection

The sample size of cubosomal dispersions was outwardly analyzed for the presence of any clogs, and homogeneity for around 1 week.[22]

Morphological studies

The prepared cubosomes morphological structure was determined using scanning electron microscopy (SEM). The size, structure, and quality of globules can all be examined with SEM. LH cubosomes were appropriately suspended in water to allow for scattering. Utilizing SEM (Model number: S-3700N), at various amplifications, the particle scattering was observed, at a reasonable speeding-up voltage.[23,24]

Analysis of particle size, zeta potential, and PDI

The Malvern molecule size analyzer was used to measure cubosomal dispersion's zeta potential and size. The polydispersity index, zeta potential, and size of diluted samples were determined by analyzing them at 25°C.[25,26]

Table 1: Coded value composition in the experimental design

Independent factors	Levels		
	−1 (Min)	0 (Medium)	+1 (Max)
Homogenization speed (rpm)	10	30	50
Sonication time (min)	5	10	15

Dependent Variables: Particle size (nm), PDI, Zeta potential (mV), % EE

Analysis of entrapment efficiency

The cubosomal dispersions were centrifuged for 30 min at 15000 rpm to assess their entrapment efficiency.[27] For drug estimation using a UV/visible spectrophotometer at 230 nm, the resulting dispersion was divided to collect the free drug solution and supernatant liquid. The EE(%) was determined by utilizing the relevant equation.[28]

Optimization and validation

Utilizing Design® expert software, an optimization strategy that made use of the desirability function, an optimized cubosome formulation that meets the prerequisites laid out earlier for EE, particle size, PDI and ZP was being emerged out.[29,30] As in the past, the expected ward factors were assessed, and the ideal definition was proposed. For every dependent variable in the modified plan, was analyzed to check whether it fits inside the 95% PI or not.[31,32] In addition, the actual and anticipated values were contrasted using the percent prediction error.

Stability studies

Accelerated stability studies were conducted for the optimized cubosomal dispersion, in accordance with ICH guidelines for storage temperature and humidity conditions. Samples were withdrawn with a time interval of 7 days and were subjected to various evaluation studies (Harmonized Triplicate Guideline).[33,34]

RESULTS AND DISCUSSION

Visual inspection

The dispersions were uniform, opaque white with no discernible aggregate. The maximum homogenization speed used for the formation of stable cubosomes was between 10 and 50 rpm and the sonication time ranged from 5 to 15 min. The ratio of GMO: P-407 in the total lipid content was determined based on observations.

Morphological studies

The SEM analysis confirmed the formation of cubosomes. The obtained micrographs demonstrated the evolved cubosomes cubic state. Figure 1 depicts the optimized (LT8) dispersion's morphology.

Investigational analysis of PDI, zeta potential, particle size, and %EE

Cubosomes containing LH were produced using the Top-down method in accordance with the QbD principles' systematic approach. The optimization data were analyzed with the Design-Expert® program (version 8.0) to generate

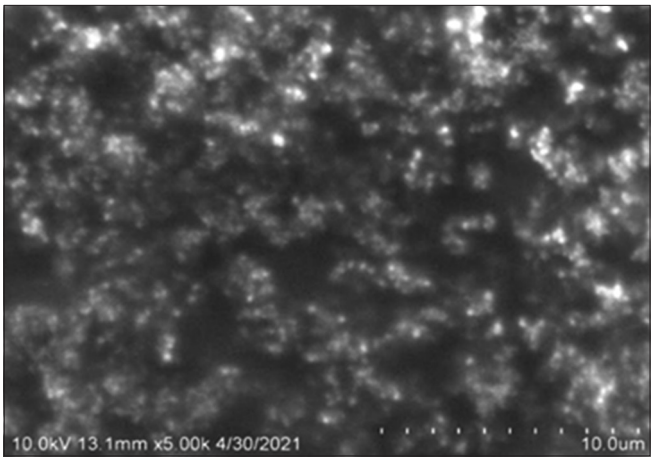

Figure 1: Scanning electron microscopy image of LT8 cubosomal dispersion

polynomial equations with additional interaction terms that linked the outcomes. Utilizing the software programming, the responses of the definition groups were evaluated for various portrayal boundaries and are depicted in Table 2.

Optimization and validation using 3^2 factorial design

Effect of independent factors on the size of particles (Y1)

As can be seen in Figures 2d and 3d, the surface response graph for the particle size parameter was narrowly skewed. The graphical plot shows that as the homogenization speed increased, the size of the molecules decreased. This was normal because as the speed increased, the pressure between the two stages decreased, which could also reduce the collection of cubosomal surface energy molecules. This is influenced by the possibility that high homogeneity could cause diminished molecule size. Surprisingly, it was observed that rising the speed of homogenization and sonication time further enhanced entanglement proficiency, despite the sonication time seeming to meaningful effect on molecule size decrease. The typical size of a molecule varied from 150.3 and 244.6 nm. However, generally high duration of sonication is favorable for the refinement of the size of the particles and also, they favor vesicular atom formation over the regular particles with a cubic shape.

Effect of independent factors on PDI (Y2)

The polydispersity indices ranged between 0.07 and 0.29. By taking into account that blended mixture settles these particles sterically and holds onto interlacing them into the cubic construction, quick homogenization and sonication time spans might make dominatingly vesicular designs with desirable PDI range as evident from Figures 2c and 3C.

The PDI measures the size distribution of nanoparticles, with lower values indicating a more uniform distribution

Table 2: The experiment's design and results of the developed formulations

Standard (Batches)	Runs	Independent factors			Dependent factors		
		Speed of homogenization (rpm)	Sonication duration (min)	Size of particles (nm)	PDI	ZP (mv)	% EE
5 (LT5)	1	0	0	196.9	0.219	24.6	67
4 (LT2)	2	−1	0	242.6	0.239	28.9	55
1 (LT1)	4	−1	−1	244.6	0.196	22	52
7 (LT3)	6	−1	1	204.9	0.177	36.3	60
8 (LT6)	7	0	1	183.3	0.078	16.5	70
9 (LT9)	8	1	1	163.7	0.88	15.1	72
3 (LT7)	9	1	−1	180.7	0.244	28	72
6 (LT8)	11	1	0	150.3	0.298	21.8	75
2 (LT4)	12	0	−1	200.7	0.258	14.1	64

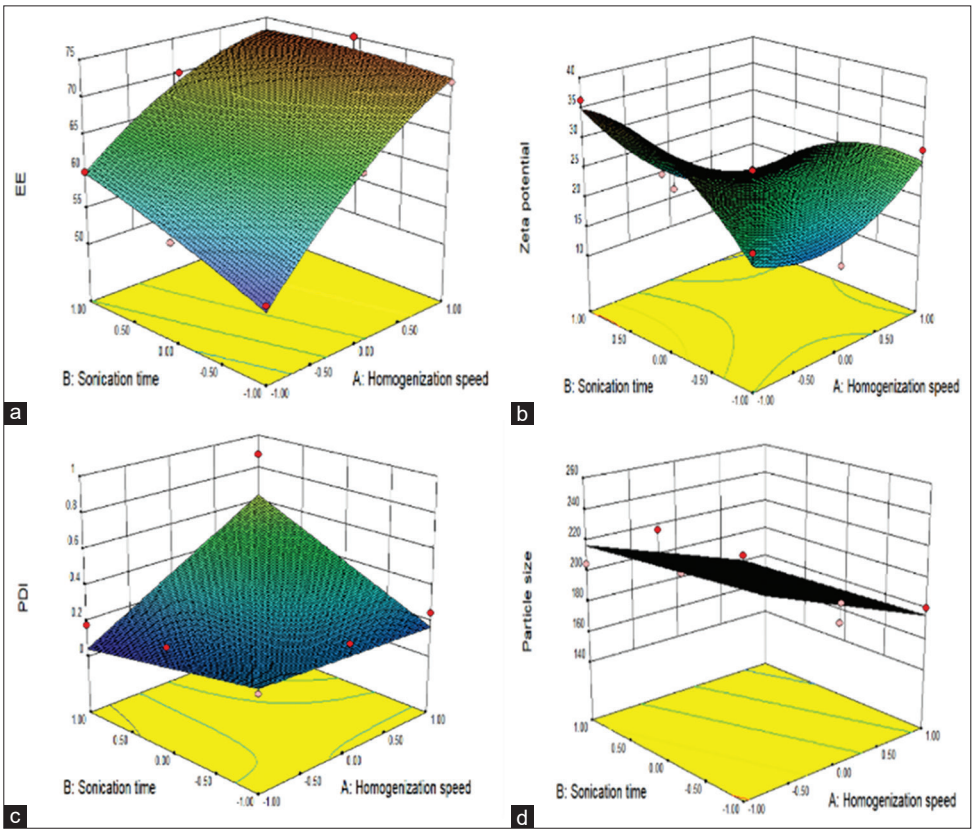

Figure 2: 3D illustration of interlinkage of the two variables interacts and the corresponding impact on the percentage of (a) EE, (b) zeta potential, (c) PDI, and (d) particulate size, respectively

and higher values indicating greater diversity in particle size. Shorter sonication periods can cause insufficient aggregate breakup, resulting in bigger, more polydisperse cubosomes (greater PDI). Longer sonication periods may lower the PDI by reducing bigger aggregates to smaller particles. Whereas, higher homogenization speeds are often more effective at reducing particle size and increasing homogeneity. However, extremely high speeds may generate shear stress or cause local heating, resulting in an increase in PDI due to aggregation. Lower homogenization speeds may result in insufficient particle size reduction, causing cubosomes to be larger and have a higher particle density index.

Therefore, longer sonication is anticipated to enhance uniformity (lower PDI) by lowering aggregate size, but only to an ideal extent. Beyond this point, the PDI could rise due to aggregation of heat-induced instability. Higher speeds are anticipated to reduce PDI by reducing particle size through increased shear

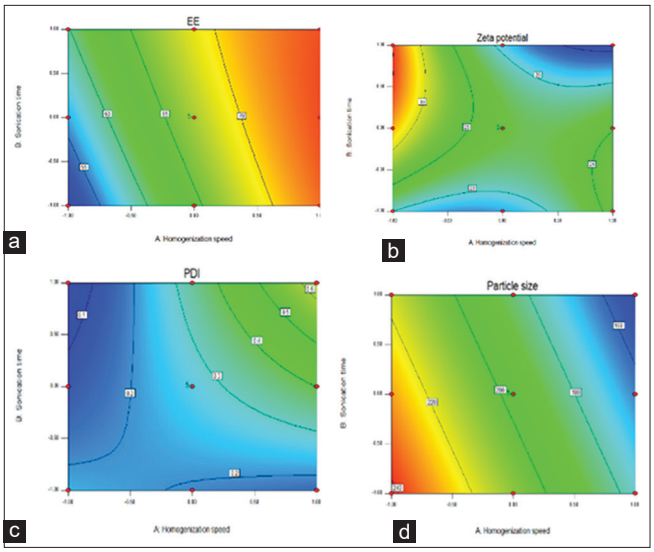

Figure 3: 2D illustration of Contour plots of the interactions between the two factors and their effects on percentage of (a) EE, (b) zeta potential, (c) PDI, (d) particulate size, respectively

pressures. However, there may be a pace at which the PDI rises due to overshearing or instability in the lipid matrix and leads to a relationship between sonication time and homogenization pace. As per the studies, prolonged sonication time paired with high homogenization speed may lead to a more significant reduction in PDI than either condition alone.

Effect of independent factors on zeta potential (Y3)

As seen in Figures 2b and 3b, the zeta potential of Lidocaine loaded varied from −14 to −36 mV. The negative value of zeta-potential confirms the stability of the particles within the formulation. It is evidently noteworthy that zeta-potential values ranging between 30 mV and −30 mV implicate complete electrostatical stability.

Increased sonication time can lower particle size, resulting in a larger surface area and ultimately more uniform particle distribution. This can influence the zeta potential by changing the surface charge. Longer sonication may alter the surface chemistry of the cubosomes, potentially improving stability by increasing or stabilizing the surface charge. On the other side, a faster homogenization speed can improve the homogeneity of cubosome dispersion and lower particle size, resulting in increased surface charge density. Faster homogenization may result in more uniform and stable cubosome formulations with more consistent zeta potential, lowering the risk of aggregation.

Effect of independent factors on EE (Y4)

After centrifuging Lidocaine HCl cubosomal nanoparticles to isolate Lidocaine HCl existing in free form, EE percent of the medication in the cubosomes was determined. The EE percentage of these Lidocaine-stacked cubosomes increased from 52% to 75% with an increase in the speed of homogenization and sonication time [Table 2]. Longer sonication times can result in smaller and more homogeneous cubosome particles, which may increase encapsulation efficiency by increasing the surface area for the active component. However, excessive sonication can break down the cubosomes or release the encapsulated substance, lowering EE. Higher homogenization rates reduce particle size, promoting the creation of stable cubosomes, which can increase entrapment efficiency by limiting active component loss during formulation, whereas, too faster speed may produce disruption or phase separation. In QbD, these parameters were optimized to balance particle size, surface charge (zeta potential), EE and overall stability of the cubosomes, with their effects resulting in effective drug loading to ensure robustness and reproducibility.

Data analysis

By applying regression analysis methods, the predicted response has been obtained as

$$Y_1 = +195.94 - 32.9*X_1 - 12.35*X_2 + 5.68*X_1*X_2 + 2.89*X_1^2 - 1.56*X_2^2$$

$$Y_2 = +0.22 + 0.030*X_1 - 0.090*X_2 + 0.16*X_1*X_2 + 0.049*X_1^2 - 0.051*X_2^2$$

$$Y_3 = +23.32 - 3.72*X_1 + 0.63*X_2 - 6.80*X_1*X_2 + 5.24*X_1^2 - 4.81*X_2^2$$

$$Y_4 = +67.14 + 8.67*X_1 + 2.33*X_2 - 2.00*X_1*X_2 - 2.48*X_1^2 - 0.48*X_2^2$$

Where Y1, Y2, Y3, and Y4 are the predicted responses and X1 and X2 are the coded values of the test variables speed of homogenization and time of sonication. Following the data input for each of the four responses, the Design-Expert® software created a quadratic model as the most appropriate model for all four dependent variables [Figures 2 and 3]. Equations illustrate that each coefficient in the polynomial equation has a value and a sign. The independent variable has the opposite effect on the result, as indicated by the negative sign. The positive sign indicates that the independent variable has a synergistic influence on the response. The numbers indicate the strength of the quantitative effects of each element represented by its coefficient, while the positive and negative signs indicate the antagonistic and synergistic effects of these linked components on the responses, respectively.

The terms "X*Y" stand for the combination of these two elements. When a model term's value is <0.05, it is considered significant; when it is larger than 0.05, it is considered inconsequential. The results demonstrated a noteworthy decrease in particle size and an enhancement in the efficiency of entrapment. The outcomes showed that the produced design space can reduce the probability of an error [Table 3].

Stability studies

The appearance, particle size, and % EE of the dispersion were measured after three months of storage at various

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

Model	R^2	Adjusted R^2	Predicted R^2	SD	%CV	*P*-value Prob>F
Y1	0.9078	0.8894	0.7930	8.69	4.41	0.0001
Y2	0.5666	0.4221	–1.0684	0.15	54.52	0.0482
Y3	0.8596	0.7593	0.0350	2.94	12.52	0.0068
Y4	0.9861	0.9762	0.8631	1.03	1.56	0.0001

Table 4: Stability investigations of cubosome dispersion (LT8)

Time frame	Size of particles (nm)	PDI	ZP (mV)	EE%
On day 1	150.3±0.78	0.298±0.39	–21.8±0.69	75±0.94
After 90 days	150±0.51	0.297±0.46	–21.8±0.39	75±0.47

temperatures and humidity levels. The outcomes showed no clear changes in apparent appearance and without any indications of precipitation [Table 4].

CONCLUSION

The present research work details the effect of homogenization speed and sonication time on Lidocaine hydrochloride-loaded cubosomes prepared by the Top-down method. 3^2experimental design utilizing DoE® programming was used to form and assess the required boundaries for the representation and assessment of LH stacked cubosomes. The 3^2factorial design was successful in predicting the speed of homogenization and sonication time within the confidence intervals required to prepare optimized formulation (LT8) which was found to be effective with respect to particle size, PDI, ZP, and %EE. A homogenization speed of 50 rpm and sonication time of 10 min were proved to be ideal for the preparation of cubosomal dispersion with optimum characterization parameters when compared with dispersions produced at different levels of processing variables.

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