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Development and Evaluation of Lidocaine Hydrochloride Cubosomes directed by QbD

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ABSTRACT: Cubosomes, which are modified cubic phase systems, are looking very promising as a method of delivering both hydrophilic and lipophilic drugs. Transdermal delivery of cubosomes is currently gaining more importance over conventional topical delivery of drugs. The proposed study aimed to produce Lidocaine hydrochloride loaded cubosomes. This study was designed to prepare various formulations of Lidocaine nano cubsomal dispersions at different concentrations of lipid and stabilizer using optimization technique. For the purpose of prolonging the duration of the local anaesthetic action, Lidocaine-loaded cubosomes were developed by bottom up method utilizing Glyceryl mono oleate and Poloxamer 407 in various ratios using the "Quality by Design" approach, 3^2 factorial design employing statistical software. Within the confidence intervals, the 3^2 statistical design was effective at forecasting the optimized formulation's composition. Surface morphology, particle size, drug content, poly dispersibility index, zeta potential, entrapment efficiency, and *in vitro* drug release studies were conducted on the prepared formulations. Several mathematical models were used to conduct and assess an *in vitro* drug release investigation. The maximal entrapment efficiency for the LH8 formulation, which was validated to have optimum cubosomes dispersion, was reported to be 78 % with vesicle size as 150 nm, Zeta potential 21.5 mV and Poly Dispersibility Index as 0.08 along with an *in vitro* drug release 80.03 % by the end of 24 hours. A stable dispersion with appreciable results of evaluation parameters of cubosomal dispersion was conferred with formulation LH8. Hence from amongst the nine formulations developed, it is concluded that LH8 is selected as the optimized dispersion to be incorporated into a gel formulation.

KEYWORDS: Lidocaine Hydrochloride; Cubosomes; Design of Experiment; Quality by design; Glyceryl mono oleate; Poloxamer

1. INTRODUCTION

Cubosomes are distinct, diminutive nano composites that are part of the persistent cubicular liquid crystal phase [1]. They are made of polar and non-polar components of polymers, lipids, and surfactants, resulting in these are referred to as amphiphilic [2]. These nanoparticles can be produced using Top down and Bottom Up techniques [3]. They are self-organized liquid crystal particles of with surfactants, lipids and water in suitable proportions [4].

Lidocaine Hydrochloride is a well-known local anesthetic used to treat post-operative pains from minor procedures as well as symptomatic relief in burns, joints, muscles, hemorrhoids, and neuralgia [5]. The half-life of Lidocaine hydrochloride (LH), which is highly water soluble, is 1.5 to 2 hours. The majority of anesthetics have a tendency to bind to plasma proteins in the blood. This influences the duration of the drug's action [6]. To ensure dermal penetration and targeting, colloidal drug carriers such as microemulsions, vesicular carriers such as liposomes and niosomes, as well as both lipid and polymeric particulate carrier systems, were developed for topical delivery of Lidocaine Hydrochloride [7].

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Cubosomes are gaining popularity in topical drug delivery due to their higher stability when compared to liposomes, which are made from phospholipids [8]. They are recently employed as an ideal system for delivery of drugs for maximum loading and permeability when compared with conventional carriers which exhibit minimal loading and permeability [9]]. Furthermore, Glyceryl mono oleate (GMO), a commonly used lipid, forms a double diamond structure in aqueous solution, having structural resemblance with stratum corneum [10]. As a result, the current study focused primarily on the preparation of Lidocaine HCl cubosomal dispersion as a promising skin retentive means for treating burns, anal fissures, haemorrhoids, and other conditions.

For formulation optimization, the Quality by Design (QbD) approach has gained importance. A minimal number of trials were necessary for the quality by design method to identify any potential relationships between various factors [11]. The relationship across formulation parameters and process variables was analyzed in the current work using the Quality by Design methodology and a statistical software design expert.

2. RESULTS

Table 1 depicts different formulation parameters adopted for this study. Mechanical stirring was used to break the cubical phase of GMO and water with poloxamer 407 as a stabilizer in order to develop cubosomal nanoparticle dispersions that were loaded with Lidocaine.

Table 1. Composition of coded values in experimental design

Independent Variables (Factor)	Coded Symbol	Levels		
		-1(Low)	0(Medium)	+1(High)
GMO (%w/w)	X_1	2	5	8
Polaxamer 407 (% w/w))	X_2	0	0.5	1
Dependent Variables	**Coded symbol**			
% EE	Y_1			
CDR %	Y_2			
Particle size nm	Y_3			
Zeta potential mV	Y_4			

The dispersions were homogenous opaque white that didn't include any apparent aggregate. In accordance to the final weight of formulated dispersion, the maximum lipid composition in the dispersion was in the range of 2-8%w/w. Observations formed the basis for the lipid to P- 407 ratio in the total lipid content.

Cubosomes containing Lidocaine were made utilizing the Bottom Up technique following systematic strategy outlined by the QbD principles. First, a thorough literature review was performed to choose the formulation procedure. The Design-Expert® programme (version 8.0) was utilized to analyze the optimization data to establish polynomial equations with additional interaction terms that link the investigated outcomes. Using DOE software, the responses of formulation batches were evaluated for various characterization parameters. Table 2 displays the cubosomes preparation findings for particle size and poly dispersity index. The average particle size falls between 150 nm and 250.7 nm.

In the current investigation, cubosomes were developed with the least amount of surface-active agent loading in the drug delivery system. As evident from Figure 1c, narrowly skewed graph of surface response was obtained for particle size parameter. The plot demonstrates that particle size decreased as the surfactant concentration was raised. This was expected because the tension between the two phases would decrease, which could also lower the cubosomes surface energy particle aggregation. It is influenced by the probability that high surfactant levels could lead to decreased particle size. Interestingly, it was observed that increasing the surfactant and stabilizer concentrations improved entrapment efficiency, despite the stabilizer appearing to have no effect on particle size reduction.

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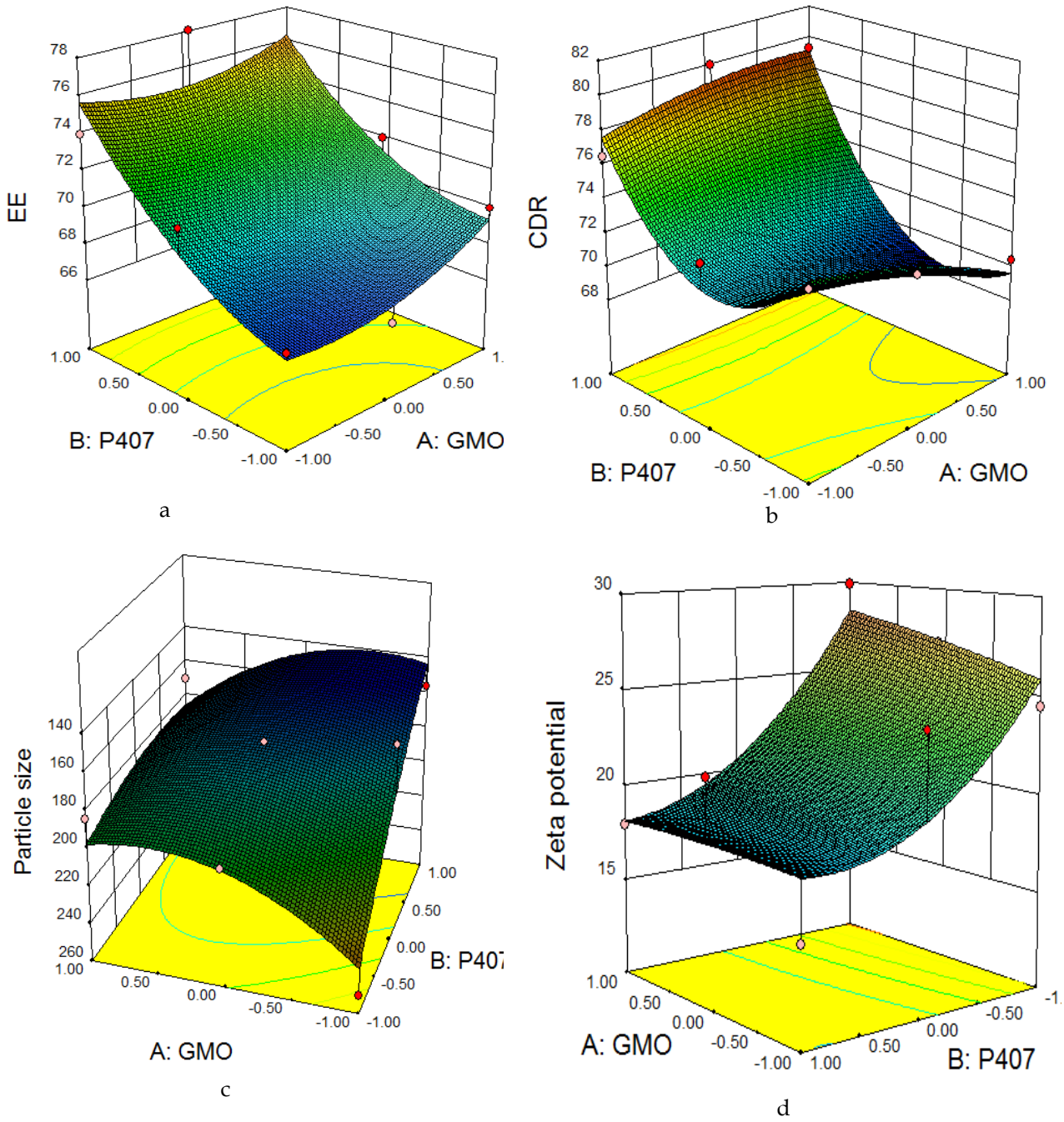

Figure 1 a,b,c,d. 3D representation of the interactions between the two factors and their effects on % EE, CDR%, particle size and zeta potential respectively.

The poly dispersity indices varied from 0.08±0.39 to 0.61±0.75. Given that mixed monoolein/poloxamer bilayers sterically stabilize the particles and prevent them from fusing into the cubic form, high concentrations of poloxamer 407 may produce predominantly vesicular structures. Although relatively high concentrations of poloxamer 407 are beneficial for the growth of minute particles, they also favour vesicular particle creation over that of the required cubic-shaped particles.

After centrifuging the drug's loaded cubosomal nanoparticles to separate the free Lidocaine HCl, the amount of EE (%) of the drug in the cubosomes was calculated. These Lidocaine-loaded cubosomes' EE (%) varied from 66% to 78% (Table 2).

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Table 2. Design of experiment and corresponding responses observed

Std (Batch)	Run	Independent Variables		Dependent Variables			
		GMO %w/w	P407 %w/w	%EE Y1	%CDR Y2	Particle size (nm) Y3	Zeta Potential (mV) Y4
3 (LH3)	1	1	-1	70	70.44	184	29.9
8 (LH8)	**2**	**0**	**1**	**78**	**80.03**	**150**	**21.5**
4 (LH4)	5	-1	0	71	72.8	157.4	24
5 (LH5)	6	0	0	69	70.9	166	19.8
9 (LH9)	7	1	1	75	79.2	170.3	17.9
6 (LH6)	8	1	0	72	68.7	213.4	19.6
7 (LH7)	9	-1	1	74	76.5	156.2	15
2 (LH2)	10	0	-1	66	72.1	197.6	27.1
1 (LH1)	11	-1	-1	67	74.01	250.7	24.5

The high EE (%) can be attributed to the drug's high degree of mobility in relation to the lipid bilayer. Furthermore, because of Lidocaine's substantial solubility in GMO and lipophilic nature, it was predicted that it would eventually get trapped inside the lipid channels of cubosomal nanoparticles. Similar to this, interpretations of the 3D response surface plot for EE designated as Figure 1a, correlated with the given observation and successfully explained how both elements have an impact and improve EE of the manufactured cubosomes. When Lidocaine-loaded cubosomes were dispersed and subjected to in vitro drug release conditions, CDR% over the period of 24 hours ranged from 68.71% to 80.03%, indicating a sustained release profile.

Data analysis

By applying regression analysis methods, the predicted response have been obtained and given as

$Y_1 = +69.41+0.83*X_1+4.00*X_2-0.50*X_1*X_2+1.05*X_1^2+1.55*X_2^2$

$Y_2 =+71.04-0.81*X_1+3.20*X_2+1.59*X_1*X_2-0.59*X_1^2+4.76*X_2^2$

$Y_3 =+166.40+0.58*X_1-25.98*X_2+20.23*X_1*X_2+17.99*X_1^2+6.39*X_2^2$

$Y_4 =+20.42+0.65*X_1-4.52*X_2-0.62*X_1*X_2-0.16*X_1^2+2.34*X_2^2$

Where Y_1, Y_2, Y_3 and Y_4 are the predicted responses and X_1, X_2 are the coded values of the test variables GMO and P407 %w/w.

The Design-Expert® programme developed two different types of models according to Predicted R^2, Adjusted R^2, and p values after the input of the data for each of the four responses (Figure 1). Each coefficient in the polynomial equation has a value and a sign, as can be seen in Equations. The negative sign indicates that the independent variable has an opposite effect on the outcome. The independent variable has a synergistic effect on the response, as indicated by the positive sign. The positive and negative signs designate the synergistic and antagonistic effects of these linked factors on the responses, respectively, while the values describe the strength of the quantitative effects of each of the elements represented by their coefficients. The combination of these two components is represented by the terms "A*B" Model terms are significant when the value is less than 0.05, and insignificant when the value is greater than 0.05. Model terms have coefficient values with p-values under 0.05 indicating they are significant. The findings showed a significant reduction in particle size and an improvement in entrapment effectiveness. The results demonstrated that the generated design space can lessen the likelihood of failure. (Table 3).

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

Model	R2	Adjusted R^2	Predicted R^2	SD	%CV	p-value Prob>F
Y_1(Quadratic)	0.8627	0.7647	-0.2428	1.63	2.3	0.0063
Y_2	0.9552	0.9232	0.5558	0.98	1.34	0.0001
Y_3	0.7586	0.5861	-1.4559	18.03	10.15	0.0393
Y_4	0.7530	0.5766	-1.2706	2.59	12.10	0.0422

The FT-Infrared spectra of individual element and their physical mixture were examined in order to identify any interactions that would cause the distinctive peaks to disappear or shift, affecting functioning

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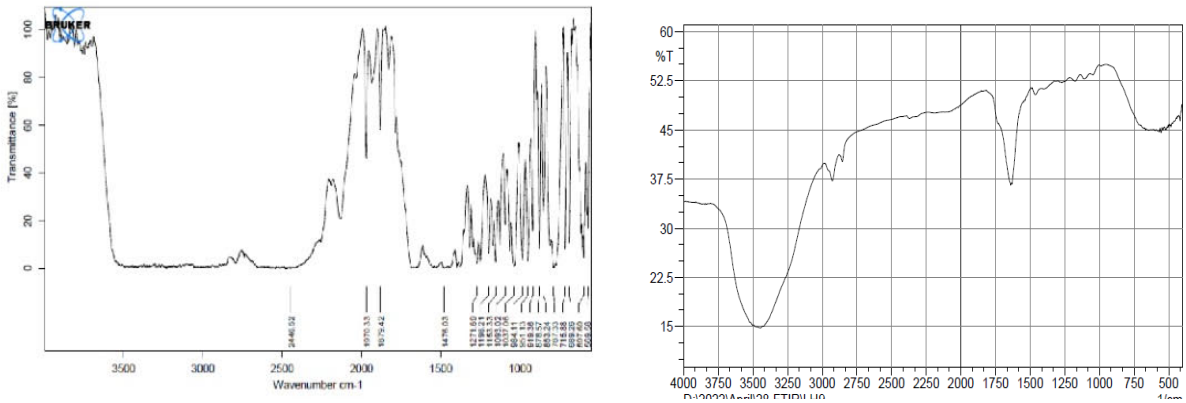
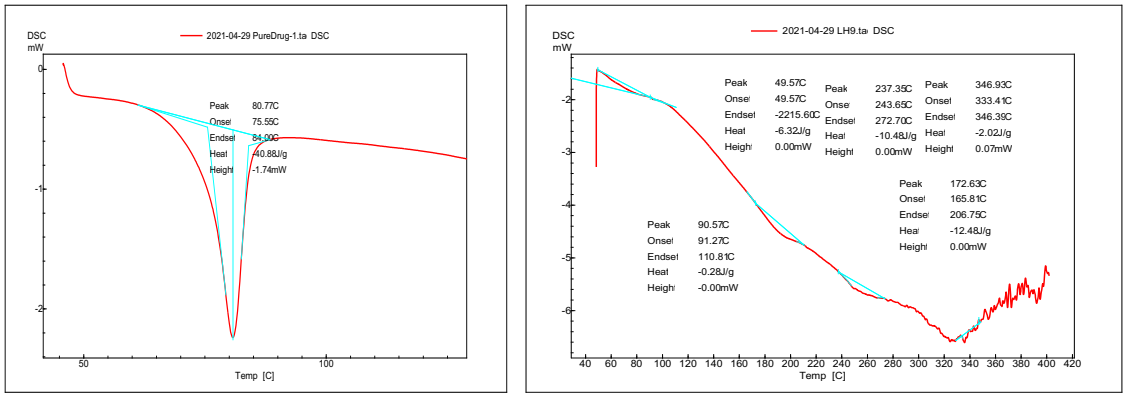
and, consequently, stability and compatibility. In Figure 2, the graphs produced for each sample are displayed. The spectral graphs' data showed that there was no potential interaction between Lidocaine and other components, either separately or together.



a b

Figure 2. FTIR spectrum of pure drug (a) and optimized formulation (b)

Similar to this, the DSC thermograms obtained for each individual ingredient and the combination were examined for the existence or absence of any other unusual peaks in the physical mixture's DSC thermogram when heated between 30 and 300 °C. Figure 3 displays the overlay of each thermogram. They concluded that there was no conflict among the different excipients in the physical composition.



a b

Figure 3. Ther*mo*grams of pure drug (a), optimized formulation (b).

Because GMOs have melting points around 35 °C, the DSC was unable to make a complete determination; however, an endothermic peak indicating its B.P. at 49 °C was visible. The thermogram showed that GMO was melting. The overlap of P- 407 and GMO endothermic peaks caused the primary endothermic peak to abruptly begin (at 237 °C), indicating that both constituents were melting, but there was no evidence of interaction between them. These findings revealed that the components used to make cubosomes were not incompatible with one another.

Despite reaching the endothermic peak at 49 °C, which may be its boiling point as indicated by the DSC thermogram, a perfect establishment was not possible due to the GMO's melting temperature of approximately 35 °C. The thermogram of Lidocaine cubosomes revealed the melting of GMO. The overlay of P-407 and GMO endothermic peaks brought a prior outset of the primary endothermic peak (at 237 °C), indicating the melting phenomena of both constituents without any evidence of interaction. These results suggested that there were no compatibility issues between the cubosome-making ingredients.

The development of cubosomes was verified by the SEM analysis. The acquired micrographs demonstrated that the cubic shape of the developed cubosomes. The morphology of the optimized (LH8) formulation is seen in Figure 4.

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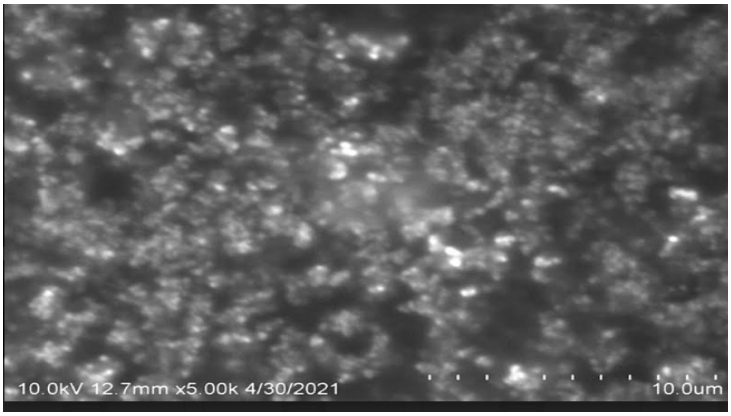

Figure 4. SEM image of optimized Lidocaine cubosomal dispersion

Using TEM, the morphology of Lidocaine cubosomal nanoparticles was examined. The image shows that the drug-loaded cubosomal nanoparticles have irregular polyangular forms without aggregation and are approximately spherical. The formation of cubic structured particles was visible in TEM images (Figure 5), demonstrating the viability of using the existing formulation parameters to create cubosomes.

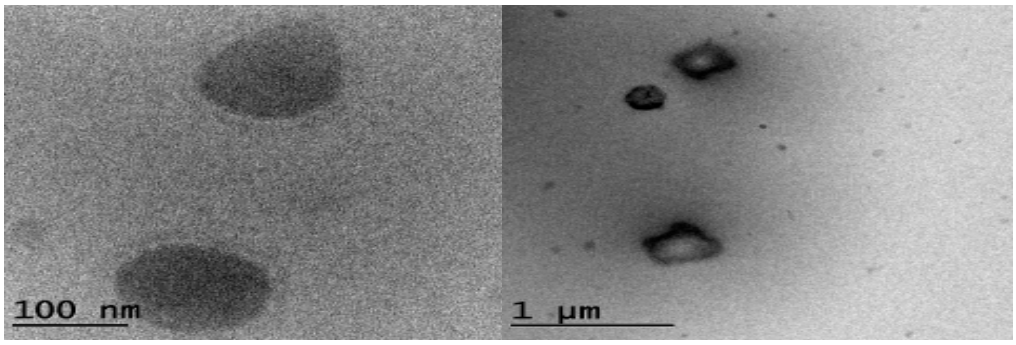

Figure 5. TEM images of optimized Lidocaine cubosomal dispersion

After 3 months of storage at varied temperature and humidity conditions, the dispersion was measured for appearance, particle size and % EE. The results showed no evident changes with regards to visible appearance and with no signs of precipitiousness (Table 4).

Table 4. Stability studies of optimized Lidocaine cubosomal dispersion

Time period	Vesicle size(nm)	PDI	Zeta potential (mV)	%EE
On day 1	150 ± 0.62	0.08 ± 0.39	-21.5 ± 0. 69	78 ± 0.94
After 90 days	150 ± 0.51	0.11 ± 0.46	-22 ± 0.39	78± 0.47

3. DISCUSSION

Table 1 depicts different formulation parameters adopted for this study. Mechanical stirring was used to break the cubical phase of GMO and water with poloxamer 407 as a stabilizer in order to develop cubosomal nanoparticle dispersions that were loaded with Lidocaine.

The dispersions were homogenous opaque white that didn't include any apparent aggregate. In accordance to the final weight of formulated dispersion, the maximum lipid composition in the dispersion was in the range of 2-8%w/w. Observations formed the basis for the lipid to P- 407 ratio in the total lipid content.

Cubosomes containing Lidocaine were made utilizing the Bottom Up technique following systematic strategy outlined by the QbD principles. First, a thorough literature review was performed to choose the formulation procedure. The Design-Expert® programme (version 8.0) was utilized to analyze

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the optimization data to establish polynomial equations with additional interaction terms that link the investigated outcomes. Using DOE software, the responses of formulation batches were evaluated for various characterization parameters. Table 2 displays the cubosomes preparation findings for particle size and poly dispersity index. The average particle size falls between 150 nm and 250.7 nm.

In the current investigation, cubosomes were developed with the least amount of surface-active agent loading in the drug delivery system. As evident from Figure 1c, narrowly skewed graph of surface response was obtained for particle size parameter. The plot demonstrates that particle size decreased as the surfactant concentration was raised. This was expected because the tension between the two phases would decrease, which could also lower the cubosomes surface energy particle aggregation. It is influenced by the probability that high surfactant levels could lead to increased particle size. Interestingly, it was observed that increasing the surfactant and stabilizer concentrations improved entrapment efficiency, despite the stabilizer appearing to have no effect on particle size reduction.

The poly dispersity indices varied from 0.08±0.39 to 0.61±0.75. Given that mixed monoolein/poloxamer bilayers sterically stabilize the particles and prevent them from fusing into the cubic form, high concentrations of poloxamer 407 may produce predominantly vesicular structures. Although relatively high concentrations of poloxamer 407 are beneficial for the growth of minute particles, they also favour vesicular particle creation over that of the required cubic-shaped particles.

After centrifuging the drug's loaded cubosomal nanoparticles to separate the free Lidocaine HCl, the amount of EE (%) of the drug in the cubosomes was calculated. These Lidocaine-loaded cubosomes' EE (%) varied from 66% to 78% (Table 2). The high EE (%) can be attributed to the drug's high degree of mobility in relation to the lipid bilayer. Furthermore, because of Lidocaine's substantial solubility in GMO and lipophilic nature, it was predicted that it would eventually get trapped inside the lipid channels of cubosomal nanoparticles. Similar to this, interpretations of the 3D response surface plot for EE designated as Figure 1a, correlated with the given observation and successfully explained how both elements have an impact and improve EE of the manufactured cubosomes. When Lidocaine-loaded cubosomes were dispersed and subjected to in vitro drug release conditions, CDR% over the period of 24 hours ranged from 68.71% to 80.03%, indicating a sustained release profile.

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Where Y_1, Y_2, Y_3 and Y_4 are the predicted responses and X_1, X_2 are the coded values of the test variables GMO and P407 %w/w.

The Design-Expert® programme developed two different types of models according to Predicted R^2, Adjusted R^2, and p values after the input of the data for each of the four responses. Each coefficient in the polynomial equation has a value and a sign, as can be seen in Equations. The negative sign indicates that the independent variable has an opposite effect on the outcome. The independent variable has a synergistic effect on the response, as indicated by the positive sign. The positive and negative signs designate the synergistic and antagonistic effects of these linked factors on the responses, respectively, while the values describe the strength of the quantitative effects of each of the elements represented by their coefficients. The combination of these two components is represented by the terms "A*B" Model terms are significant when the value is less than 0.05, and insignificant when the value is greater than 0.05. Model terms have coefficient values with p-values under 0.05 indicating they are significant. The findings showed a significant reduction in particle size and an improvement in entrapment effectiveness. The results demonstrated that the generated design space can lessen the likelihood of failure. (Table 2, 3).

The FT-Infrared spectra of individual element and their physical mixture were examined in order to identify any interactions that would cause the distinctive peaks to disappear or shift, affecting functioning and, consequently, stability and compatibility. In Figure 2, the graphs produced for each sample are displayed. The spectral graphs' data showed that there was no potential interaction between Lidocaine and other components, either separately or together.

Similar to this, the DSC thermograms obtained for each individual ingredient and the combination were examined for the existence or absence of any other unusual peaks in the physical mixture's DSC thermogram when heated between 30 and 300 °C. Figure 3 displays the overlay of each thermogram. They concluded that there was no conflict among the different excipients in the physical composition.

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Because GMOs have melting points around 35 °C, the DSC was unable to make a complete determination; however, an endothermic peak indicating its B.P. at 49 °C was visible. The thermogram showed that GMO was melting. The overlap of Poloxamer- 407 and GMO endothermic peaks caused the primary endothermic peak to abruptly begin (at 237 °C), indicating that both constituents were melting, but there was no evidence of interaction between them. These findings revealed that the components used to make cubosomes were not incompatible with one another.

The maximal entrapment efficiency was attained for the LH8 formulation and was reported to be 78%. On comparision of the vesicle size, zeta potential, poly dispersity index and *in vitro* drug release patterns of all formulated cubosomal dispersions, LH8 depicted maximum values among all attained parameters, which includes vesicle size of 150 nm, Zeta potential of 21.5 mV and Poly Dispersity Index as 0.08 along with an *in vitro* drug release of 80.03% by th end of 24 hour. Appreciable values of LH8 enable it to be identified as on optimized cubosomal dispersion.

The development of cubosomes was verified by the SEM analysis. The acquired micrographs demonstrated that the cubic shape of the developed cubosomes. The morphology of the optimized (LH8) formulation is seen in Figure 4.

Using TEM, the morphology of Lidocaine cubosomal nanoparticles was examined. The image shows that the drug-loaded cubosomal nanoparticles have irregular polyangular forms without aggregation and are approximately spherical. The formation of cubic structured particles was visible in TEM images (Figure 5), demonstrating the viability of using the existing formulation parameters to create cubosomes.

After 3 months of storage at varied temperature and humidity conditions, the dispersion was measured for appearance, particle size and % EE. The results showed no evident changes with regards to visible appearance and with no signs of precipitiousness (Table 4).

4. CONCLUSION

The current research work details about QbD driven formulation, fabrication and evaluation of Lidocaine Hydrochloride loaded cubosmes prepared by bottom up method. 3^2 statistical design employing DoE® software was utilized to formulate and evaluate the needed parameters for characterization and evaluation of Lidocaine loaded cubosomes. Within the confidence intervals, the 3^2 factorial design model was successful in predicting the optimization formulation's composition. From the results of Surface morphology, particle size, drug content, poly dispersibility index, zeta potential, entrapment efficiency, and *in vitro* drug release studies of Lidocaine cubosomal formulations, LH8 formulation was validated to have optimum cubosomes dispersion and was found to be effective in terms of *In Vitro* and *Ex Vivo* release of drug when compared amongst all other prepared cubsomal dispersion of Lidocaine. Further, this formulation could be used to be incorporated into gel and produce a successful nano cubosomal gel.

5. MATERIALS AND METHODS

The supplier of Lidocaine HCl was Yarrow Chem Products in Mumbai. Poloxamer 407 (P-407) was obtained from Daewoong Pharmaceuticals in Hyderabad as a kind gift sample. Triethanolamine, methylparaben, and glyceryl monooleate (GMO) were all acquired from Finar Chemicals in Mumbai.

5.1. Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry compatibility studies (DSC)

To assess likely interactions between the single ingredient and the mix, FTIR spectroscopy and DSC studies were utilized to look at individual and actual blends in different combinations [12, 13]. For DSC examination, 40 µL standard aluminum crucibles that were vacant and pleated were warmed to temperatures somewhere in the range of 30 and 300 °C at a heating pace of 10 °C/min utilizing an intensity DSC-60. The crucibles were then filled with single or mixed constituents (Mettler Toledo) [14].

5.2. Preparation of calibration curve in 7.4 pH phosphate buffer

In a volumetric flask, 100 mg of Lidocaine HCl was dissolved in phosphate buffer (PB; pH 7.4), and the volume was made upto 100 mL with PB to produce 1000 µg/mL. After making serial dilutions their respective absorbance was measured using a UV/visible spectrophotometer at 230 nm, and a calibration curve was generated [15].

5.3. Experimental design

In order to achieve systemic optimization of cubosomes containing Lidocaine hydrochloride, a 3^2 factorial design of the experiment was used, and 9 experiments were developed (each trial was conducted

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three times). Each of the chosen critical factor levels is mathematically denoted as "-1," "0," or "+1" based on the low, middle, and high levels of the critical material attributes utilized in forming the working knowledge space [16]. Utilizing DOE® software (Stat-Ease Inc.), the responses were assessed, and statistically validated equations were derived for the development of an optimized batch for further investigation [17]. The development of an improved batch for further research required statistically proven equations, which were subsequently derived. The actual and coded levels of the variables utilized in the design are shown in Table 1 together with the independent and dependent variables. The independent variables are designated as GMO (X1) and Polaxamer 407 (X2).

In contrast, the dependent variables in this study are designated as entrapment efficiency (Y1), %Cumulative drug release (Y2), Particle size (Y3), and Zeta Potential (Y4). A second-order polynomial equation assesses the significance of the dependent variable (Y) to the independent variables (X) [18]. Following is a description of the model: $Y = \beta 0 + \beta 1 X_1 + \beta 2\ X_2 + \beta 12 X_1 X_2 + \beta 11 X_1^2 + \beta 22 X_2^2$ Where Y is the response, X1 and X2 are the independent factors, $\beta 1$, $\beta 2$, $\beta 12$, $\beta 11$, $\beta 22$ are the coefficients. The interaction terms show how the response modifies when two factors are simultaneously adjusted [19].

5.4. Preparation of Lidocaine HCl loaded cubosomes using bottom-up technique

In this method, lipid (GMO) and hydrotope (alcohol) were added at different concentrations and homogenized at low energy before adding stabilizer (Poloxamer-407) with excess water by vortexing at the same low energy input [20]. Precipitation in the form of cubosomes is ultimately formed as a result [21]. The resulting dispersions were stored in closed vials at room temperature before being evaluated.

5.5. Characterization of Lidocaine hydrochloride loaded cubosomes for Surface morphology, Particle size, poly dispersibility index and entrapment efficiency (%).

5.5.1. Visual Examination

For about a week, the cubosomal dispersions were visually inspected for color, the presence of any aggregates, and homogeneity [22]. An excellent dispersion has a milky white consistency, no particles, and no clumps.

5.5.2. Structural interpretation by scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to determine the morphological structure of the prepared cubosomes [23]. SEM is used to examine globule size, structure, quality, and density [24]. Dilute dispersion of LH cubosomes was obtained by suitably suspending in water. Under SEM (Hitachi S-3700 N model), the diluted dispersion was observed at various magnifications and a suitable accelerating voltage.

5.5.3. Structural interpretation by transmission electron microscopy (TEM)

500 µL of the cubosomal dispersion was collected and suspended in water up to 2 mL to attain diluted form of cubosomal dispersion [25]. The dilute LH dispersion was cast onto 200 mesh carbon-coated grids and observed under TEM using the Philips.

5.5.4. Particle Size Analysis, Poly dispersibility index and Zeta Potential

Analysis of particle size was carried out using a Malvern Zetasizer at 25 °C temperature with a dispersant refractive index of 1.330 [26] to determine size, poly dispersibility index (PDI), and zeta potential values with an investigated quantity of prepared cubosomal dispersion which was further diluted to ten times with water [27].

5.5.5. Lidocaine HCl Entrapment Efficiency in cubosomes

The dispersion was centrifuged at 15000 RPM for 30 minutes (Remi Motors, India), later the free drug and the cubosomal dispersion were separated in order, to determine the entrapment efficiency of Lidocaine loaded cubosomes. The formulated dispersion's supernatant containing Lidocaine was diluted and analyzed spectrophotometrically at wavelength of 230nm, and the entrapment efficiency (%) was calculated using the formula [28]- EE%= (amount of entrapped drug)/(total amount of drug) x 100

5.6. *In vitro* drug release study

By using Franz diffusion cells with donor and receiver compartments in an in *vitro* study, the rate of drug release and the duration of its action were assessed [29]. A magnetic stirrer with a heating mantle set at 37°C±0.5°C was used to support this system. Release studies were conducted by placing 7.4 pH phosphate buffer (PB) in the receptor cell using gelatin membrane and cubosomal dispersion being placed

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on the membrane, which was in contact with receptor medium [30]. Aliquot of the samples were taken from the receptor cell at regular time intervals (i.e., 1, 2, 3, 4…12 h). To ensure sink conditions, the sample was removed and refilled immediately with 7.4 pH PB. The samples' drug content was examined using a UV spectrophotometer at 230 nm wavelength.

5.7. *Ex vivo permeability studies*

Skin from a goat's abdomen was taken. After removing the hair, subcutaneous fat, and other visceral tissue, the skin was gently cleansed [31]. The skin was warmed to room temperature before being mounted with the stratum corneum facing the donor chamber that was filled with pH 7.4 PB Saline and sandwiched between the two compartments of the Franz diffusion cell [32]. The cell was placed on a magnetic stirrer that was set to 37°C. The samples were aliquoted at regular intervals (i.e. 1, 2, 3, 4, 12 h) while maintaining the sink conditions, and they were then examined using a UV-visible spectrophotometer at 230 nm.

5.8. Validation and Optimization

Using Design® expert software, an optimal cubosomal formulation that satisfies the previously specified requirements on the entrapment efficiency, CDR, particle size, zeta potential, and PDI was developed using an optimization technique using the desirability function [33]. As before, the required dependent variables were evaluated, and the optimum formulation was suggested. For each dependent variable in the optimized formulation, the actual value was examined to see if it fit within the 95% PI or not. Additionally, using percent prediction error, the actual values and anticipated values were compared.

5.9. Statistical investigation

The statistical evaluation of the in-vitro trials was conducted using one-way analysis of variance (ANOVA) [34]. If P 0.05, the mean differences between the samples were deemed significant.

5.10. Stability studies

The optimized Lidocaine HCl cubosomal dispersion was stored until disrupted at varied temperature and humidity conditions and tested at 40 ^{0}C ± 2 ^{0}C, 60% ± 5% relative humidity and at 4 ^{0}C in the refrigerator [35]. After 3 months of storage at varied humidity conditions, the dispersion was measured for appearance, particle size and %EE.

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REFERENCES

[1] Tekade AR, Avhad GD. A review on cubosome: a novel approach for drug delivery. Int. J. Pharm. Sci. Res. 2022; 13 (2): 579-588. https://doi.org/10.13040/IJPSR.0975-8232.13(2).579-588.

[2] Sherif AG, Omar HG, Hamdy A. Cubosomes: composition, preparation and drug delivery applications. J. Adv. Biomed. Pharm. Appl. 2020; 3:1-9. https://dx.doi.org/10.21608/jabps.2019.16887.1057.

[3] Nilesh RR, Pramod BK. Cubosomes: a vehicle for delivery of various therapeutic agents. MOJ Toxicol. 2018; 4(1): 19-21. https://doi.org/10.15406/mojt.2018.04.00083.

[4] Kaur SD, Singh G, Singh G, Singhal K, Kant S, Bedi N. Cubosomes as Potential Nanocarrier for Drug Delivery: A Comprehensive Review. J. Pharm. Res. Int. 2021; 33(31B): 118-135. https: //doi.org/10.9734/jpri/2021/v33i31B31698.

[5] Rajani T, Umasankar K, Rao GSNK, Rajasekhar A, Chakravarthy G. Alekhya M. Fabrication and evaluation of Lidocaine Hydrochloride cubosomes. Res. J. Pharm. Technol. 2021;14(10): 5288-5302. https: //doi.org/ 10.52711/0974-360X.2021.00922.

[6] Zhao X, Sun Y, Li Z. Topical anesthesia therapy using lidocaine-loaded nanostructured lipid carriers: tocopheryl polyethylene glycol 1000 succinate-modified transdermal delivery system. Drug Des, Devel. Ther. 2018; 12: 4231–4240. https://doi.org/10.2147%2FDDDT.S187177.

[7] Soraya B, Saeed G, Soodabeh D, Maryam K, Hamed H. Nanoethosomes for Dermal Delivery of Lidocaine. Adv. Pharm, Bull. 2015; 5(4): 549-556. https://doi.org/10.15171%2Fapb.2015.074.

[8] Dhadwal A, Sharma DR, Pandit V, Ashawat MS, Kumar P. Cubosomes: A Novel Carrier for Transdermal Drug Delivery. J. Drug. Deliv. Ther. 2020; 10(1):123-130. https://doi.org/10.22270/jddt.v10i1.3814.

[9] Lakshmi PY, Pavan KJ, Mohan VM, Anand A. Cubosomes: A novel drug delivery system. Int. J. Res. Ayurveda Pharm. 2020; 11 (5): 198-204. https://doi.org/ 10.7897/2277-4343.1105169.

[10] Anan Y, Huiling M. Recent advances in drug delivery applications of cubosomes, hexosomes, and solid lipidnanoparticles. Acta. Pharm. Sin. B. 2021; 11(4): 871–885. https://doi.org/10.1016/j.apsb.2021.02.013.

[11] Tejashree W, Dabholkar N, Gorantla S, Rapalli VK, Singhvi G. Quality by design in the formulation and optimization of liquid crystalline nanoparticles (LCNPs): A risk based industrial approach. Biomed. Pharmacother. 2021; 141: 1-15. https://doi.org/10.1016/j.biopha.2021.111940.

[12] Fahima H, Mohamed N, Mohamed Y. Formulation and Characterization of Cubosomes Containing REB for Improvement of Oral Absorption of the Drug in Human Volunteers. J. Adv. Res. 2018; 2(2): 95-103. https://dx.doi.org/10.21608/aprh.2018.5828.

[13] Marzuka K, Mohammed HD. Development of inhalable cubosome nanoparticles of Nystatin for effective management of Invasive Pulmonary Aspergillosis. Istanbul J. Pharm. 50 (3): 224-237. https: //doi.org/ 10.26650/IstanbulJPharm.2020.0006.

[14] Yong T, Jian L, Jin Z, Na Z, Hong Z, Lili L, Lingvi S. Folic acid tragetted etoposide cubosomes for Theranostic application of cancer cell imaging and therapy. Med. Sci. Monit. 2017; 23: 2426–2435. https://doi.org/10.12659/msm.904683.

[15] Thoutreddy R, Gullapudi M, Bonepally R. Fabrication and evaluation of Dexamethasone loaded cubosomes. Res. J. Pharm. Technol. 2020; 13(2): 709-714. https://doi.org/10.5958/0974-360X.2020.00135.3.

[16] Ananda K. CH, Madhubabu A, Padmanabha RA, Vasudha B, Vinod KY. Design, Formulation, In-Vitro and Ex-Vivo Evaluation of Atazanavir Loaded Cubosomal Gel. Biointerface Res. Appl. Chem. 2021; 11(4): 12037-12054. https://doi.org/10.33263/BRIAC114.1203712054.

[17] Sriram P, Yamsani MR, Yamsani SK. Use of Box- Behnken Experimental design for Optimization of process variables in Iontophoretic delivery of Repaglinide. J. Young Pharm., 2016; 8(4): 35-355. https: //doi.org/ 10.5530/jyp.2016.4.10

[18] Hadel AE, Areej HA. Nanostructured liquid crystalline formulation as a remarkable new drug delivery system of anti-epileptic drugs for treating children patients. Saudi Pharm. J. 2018; 26(6): 790-800. https://doi.org/10.1016/j.jsps.2018.04.004.

[19] Rapalli VK, Banerjee S, Khan S, Jha PN, Gupta G, Dua K, Hasnain MS, Nayak, AK, Dubey SK, Singhvi G. QbD-driven formulation development and evaluation of topical hydrogel containing ketoconazole loaded cubosomes. Mater Sci Eng C Mater Biological Appl.2021; 119*:* 111548.
https: //doi.org/ 10.1016/j.msec.2020.111548.

[20] Saffron JB, Elly KB, Karren JE. Bottom up cubosome synthesis without organic solvents. J. Colloid Interf. Sci. 2021; 601: 98-105. https://doi.org/10.1016/j.jcis.2021.05.072.

[21] Hassaan U, Habibah AW, Amirah MG, Hafsa T. Waqas A. Cubosomes: Design, Development and Tumour Targeted Drug Delivery Applications. Polym. J. 2022; 14(15): 1-18. https://doi.org/10.3390/polym14153118.

[22] Vaishali T, Vaishali K, Lalji B, Mukesh G, Tejal G, Nirav P. Development and characterization of novel hydrogel containing anti-microbial drug for treatment of burns. Int. J. Pharm. Res. 2016; 6(3): 158-163. https://doi.org/10.4103/2230-973x.187343.

[23] Pavani S, Mounika K, Naresh K. Formulation and evaluation of Acyclovir Microspheres. Iraqi. J. Pharm. Sci. 2018; 27(1): 1-7. https://doi.org/10.31351/vol27iss1pp1-7

[24] Bhaskar K, Sunil J. Satveer J. Formulation and evaluation of Resveratrol loaded cubosomal nanoformulation for topical delivery. Curr. Drug Deliv. 2021; 18(5): 607-619.
http://doi.org/10.2174/1567201817666200902150646.

[25] Omar SM, Ismail A, Hassanin KD, Dawoud SH. Formulation and Evaluation of Cubosomes as Skin Retentive System for Topical Delivery of Clotrimazole. J. Adv. Pharm. Res. 2019; 3 (2), 68-82.
https: //doi.org/ :10.21608/APRH.2019.9839.1079.

[26] Peng X, Zhou Y, Han K, Qin L, Dian L, Li G, Pan X, Wu C. Characterization of cubosomes as a targeted and sustained transdermal delivery system for capsaicin. Drug Des. Devel. Ther. 2015; 9:4209-4218. https://doi.org/10.2147%2FDDDT.S86370.

[27] Sadeq AS, Nidhal KM. Optimiztion and *In Vitro* evaluation of the release of class II drug from its nanocubosomal dispersion. Int. J. App. Pharm. 2019, 11(2): 86-90. https://doi.org/10.22159/ijap.2019v11i2.30582.

[28] Flak DK, Adamski V, Nowaczyk G, Szutkowski K, Synowitz M, Jurga S, Held-Feindt J. AT101-Loaded Cubosomes as an Alternative for Improved Glioblastoma Therapy. Int J Nanomedicine. 2020; 5(15): 7415-7431. https: //doi.org/10.2147/IJN.S265061.

[29] Mohamed N, Mohamed KG & Ahmed A, *In Vitro* and *In Vivo* evaluation of cubosomes containing 5-Fluorouracil for liver targeting, Acta Pharmaceutica Sinica B. 2015, 5(1): 79-88. https://doi.org/10.1016%2Fj.apsb.2014.12.001.

[30] Hanan ME, Alia B, Nevine SA, Nihal ES. Cubosomes as Oral Drug Delivery Systems: A Promising Approach for Enhancing the Release of Clopidogrel Bisulphate in the Intestine. Chem. Pharm. Bull. 2018; 66(12): 1165-1173. https: //doi.org/ 10.1248/cpb.c18-00615.

[31] Alaa EE, Salwa S, Mahmoud G. Formulation and evaluation of cubosomes drug delivery system for treatment of Glaucoma: Ex vivo permeation and in vivo pharmacodynamic study. J. Drug Deliv. Sci. Technol. 2019; 52. http://dx.doi.org/10.1016/j.jddst.2019.04.036.

[32] Hoda ET, Islam AK, Heba MS, Novel cubosome based system for ocular delivery of Acetazolamide, Drug Deliv. 2021; 1:2177-2186. https://doi.org/10.1080%2F10717544.2021.1989090.

[33] Muheem A, Shakeel F, Warsi M, Jain G, Ahmad F, A Combinatorial Statistical Design Approach to Optimize the Nanostructured Cubosomal Carrier System for Oral Delivery of Ubidecarenone for Management of Doxorubicin-Induced Cardiotoxicity. J. Pharm. Sci. 2017; 106(10): 1-17. https://doi.org/10.1016/j.xphs.2017.05.026.

[34] Hosny KM. Nanosized Cubosomal Thermogelling Dispersion Loaded with Saquinavir Mesylate to Improve Its Bioavailability: Preparation, Optimization, *in vitro* and *in vivo* Evaluation. Int. J. Nanomedicine. 2020; 15:5113-5129. https://doi.org/10.2147/IJN.S261855.

[35] Ankit A, Prakash G, Vinay CH. Determination of Mucoadhesive behaviuor of Timolol maleate liquid crystalline cubogel by different Techniques. Asian J. Pharm. Res. 2019; 9(1): 7-11. https://doi.org/10.5958/2231-5691.2019.00002.9