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# Formulation, Development and Evaluation of Topiramate Loaded Niosomes for the Treatment of Epilepsy

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***Abstract:*** *Topiramate (TPM) is an anti-epileptic drug used in the treatment of epilepsy and seizures. The study was designed with three aims. First, to enhance the solubility and bioavailability of BCS class III drug TPM; second, to ease administration of the formulation to the epileptic patient, during an attack, and third, to decrease the dose of drug for enduring treatment. Formulation of TPM niosomes was optimized by changing the concentration of Tween, Labrafil and cholesterol using response surface design. Further the TPM niosomes were prepared by using ether injection method. The formulation was then evaluated for vesicle size, entrapment efficiency and in-vitro drug release study. FTIR and DSC studies were performed for pure drug and optimized batch. The vesicle size of the optimized batch was found to be 0. 35 nm. The %entrapment efficiency and %drug release of optimized batch was found to be 94.64% and 92.027% respectively. From the present study it can be concluded that the developed niosomes of TPM has shown great potential in treatment of epilepsy.*

***Keywords****: Niosomes, Tween 80, Labrafil, Ether injection method*

## I. Introduction

Niosomes are multilameller vesicular structure of nonionic surfactants, parallel to liposomes and are composed of non-ionic surfactant instead of phospholipids which are the components of liposomes. [1,2]. The noisome is ended of a surfactant bilayer with its hydrophilic ends bare on the outside and inside of the vesicle while the hydrophobic chains face each other within the bilayer. The vesicle is prepered of a bilayer of non-ionic surface active agents and hence the name niosomes. A vehicle for drug formulation, they may reduce the systemic toxicity of clinically important anticonvulsant agents. Niosomes may improve therapeutic index by restricting drug effects to target cells5 [3, 4] Niosomal drug delivery has a number of potential advantages over conventional dosage forms. Niosomes are potentially applicable to many pharmacological agents for their action against various diseases. [5]
Topiramate (TPM) is a new antiepileptic drug that acts through blocking of sodium channels, enhancing GABA-induced influx of chloride, and inhibiting kainate/AMPA glutamate receptors. [6,7] In Europe, TPM is indicated as monotherapy in adults and children aged six years and above with newly diagnosed epilepsy who have generalized tonic-clonic seizures or partial (focal) seizures with or without secondarily generalized seizures[8]. Epilepsy can be defined as the propensity to have seizures, a seizure being the consequence of excessive but synchronous discharge of cortical neurons. Epilepsy affects approximately 1% of the world population at any one time (about 50 million people world-wide) and is one of the most common serious neurological conditions. Topiramate a structurally novel compound was licensed in the UK in 1995 and is now licensed world-wide for adjunctive therapy in patients with intractable partial epilepsy. [9]

## II. Material And Methods

***Material***
Topiramte was obtained as gift sample from GlaneMark Pharmaceuticals Pvt. Ltd. Mumbai (India). Poloxyethylene sorbitan monolaurate (Tween 20), Polyoxyethylene sorbitan monostearate (Twen 60), Polyoxyethylene sorbitan monoleate (Tween 80), sorbitan monolaurate , Labrafil 2125 cs Methanol and Diethyl ether were purchased from S.

D. Fine Chemicals, Mumbai. Cholesterol was purchased from Loba Chemicals; Mumbai. The phosphate buffer saline (ph 7.4) was prepared as described in Indian Pharmacopoeia (1996). All other solvents and reagents used for the study were of analytical grade.

***Method***

***Ftir (Fourier Transform Infrared Spectroscopy) Studies***

IR spectroscopy also has its application in studies of drug – excipient interaction, contaminant analysis, etc. IR spectrum with the highest quality is acquired by KBr (pellet) method. Compatibility study of the drug with the excipients was determined by using FTIR. The sample powder of drug, excipients and mixture were prepared and placed on the glass plate and application of the infrared beam to record the spectra. The mixture spectra were compared with that of the original spectra. [10]

***DSC (Differential Scanning Calorimetry)***

DSC was performed using DSC 60A calorimeter to study the thermal behavior of drug alone and prepared topiramate niosomal dispersion.The samples were excited in hermetically conserved aluminum pans under nitrogen flow (30ml/min) at the scanning rate of $100^0$C/min from $500^0$C to $3000^0$C.

***Formulation of Niosomes***

***Ether Injection Method***

Niosomes containing Topiramate were prepared by modified ether injection technique using non-ionic surfactants (Tween 80 and Labrafil) and cholesterol at different ratios. Cholesterol and surfactants were assorted in 6 ml diethyl ether mixed with 2 ml methanol, containing weighed quantity of Topiramate. The obtained solution was slowly injected by using micro syringe, at a rate of 1 ml/min via 14-gauge needle into 15 ml of aqueous phase maintained at temperature 60°C.9 The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60–65°C. Then, the formulations were sonicated three times at 50 Hz in a bath-sonicator (Ralsonics model RP 120, Mumbai, India) for 15 min with a 5 min interval in between. Different batches of niosomes were prepared in order to select an optimized formula as per the general method described above. [11]

***Experimental Design For Niosomes***

The optimization study for given method was performed by using response surface design (design expert, version 12). Concentration of surfactant (tween 80, labrafil), concentration of cholesterol, sonication time, and type of surfactant was chosen as independent variables. On the other side, particle size (Y1), % entrapment efficiency (Y2), % drug release (Y3) were selected as dependant variables

## III. Optimization of Niosomes

***Independent Variables Selected For Optimization Studies Include***

| **Independent variables** | **Parameters** | **Levels** | |
|---|---|---|---|
| | | **-1** | **+1** |
| **$X_1$** | Conc of surfactant | 1 molar conc. | 3 molar conc. |
| **$X_2$** | Conc of cholesterol | 1 molar conc. | 3 molar conc. |
| **$X_3$** | Drug ratio | 1:1 | 1:3 |
| **$X_4$** | Type of surfactant | Tween 80 | Labrafil |
| **$X_5$** | Sonication time (Min) | 0 | 30 |
| **$X_6$** | RPM | 100 | 500 |

Table : Independent variables selected for optimization studies

***Dependent Variables Selected for Optimization Studies Includes***

| | | |
|---|---|---|
| $Y_1$ | Entrapment Efficiency | Response 1 |
| $Y_2$ | Particle size | Response 2 |
| Y3 | Drug Release | Response 3 |

Table : Dependent variables selected for optimization studies

***Formulation Of Batch According To Factorial Design***

| Run | Factor 1 Concentration of Surfactant (mg) | Factor2 (Concentration of Cholesterol (mg) | Factor 3 (Sonication time (min) | Factor4 Type of surfactant |
|---|---|---|---|---|
| 1 | 2 | 2 | 18.5 | Tween80 |
| 2 | 1 | 3 | 30 | Tween 80 |
| 3 | 3 | 3 | 30 | Tween 80 |
| 4 | 2 | 2 | -0.840618 | Tween 80 |
| 5 | 3 | 3 | 7 | Tween 80 |
| 6 | 2 | 2 | 18.5 | Labrafill |
| 7 | 0.318207 | 2 | 18.5 | Tween 80 |
| 8 | 1 | 1 | 7 | Labrafill |
| 9 | 2 | 2 | 18.5 | Labrafill |
| 10 | 2 | 2 | 37.8406 | Tween 80 |
| 11 | 3 | 1 | 30 | Labrafill |
| 12 | 1 | 3 | 7 | Tween 80 |
| 13 | 3 | 1 | 7 | Labrafill |
| 14 | 2 | 2 | 18.5 | Labrafill |
| 15 | 3.68179 | 2 | 18.5 | Tween 80 |
| 16 | 1 | 1 | 30 | Tween 80 |
| 17 | 2 | 3.68179 | 18.5 | Labrafill |
| 18 | 2 | 2 | 18.5 | Labrafill |
| 19 | 2 | 2 | 18.5 | Labrafill |
| 20 | 2 | 2 | 18.5 | Tween 80 |
| 21 | 1 | 3 | 30 | Labrafill |
| 22 | 2 | 2 | -0.840618 | Labrafill |
| 23 | 3 | 1 | 7 | Tween 80 |
| 24 | 0.318207 | 2 | 18.5 | Labrafill |
| 25 | 3 | 3 | 7 | Labrafill |
| 26 | 3.68179 | 2 | 18.5 | Labrafill |
| 27 | 2 | 2 | 18.5 | Tween 80 |
| 28 | 2 | 2 | 18.5 | Tween 80 |
| 29 | 2 | 0.318207 | 18.5 | Labrafill |

| | | | | |
|---|---|---|---|---|
| 30 | 2 | 2 | 18.5 | Labrafill |
| 31 | 1 | 3 | 7 | Labrafill |
| 32 | 2 | 2 | 18.5 | Tween 80 |
| 33 | 2 | 3.68179 | 18.5 | Tween 80 |
| 34 | 1 | 1 | 30 | Labrafill |
| 35 | 3 | 1 | 30 | Tween 80 |
| 36 | 2 | 2 | 18.5 | Tween 80 |
| 37 | 1 | 1 | 7 | Tween 80 |
| 38 | 3 | 3 | 30 | Labrafill |
| 39 | 2 | 0.318207 | 18.5 | Tween 80 |
| 40 | 2 | 2 | 37.8406 | Labrafill |

Table no: Formulation of Batches

## IV. Characterization of Sonicated Vesicles

***Determination Of Vesicle Size***

Vesicle size of the niosomes was determined by using digital microscope A drop of niosomal dispersion was spread on clean glass slide. The cover slip was placed over the slide having drop of noisome dispersion and evaluated the average vesicle size and shape by an ordinary optical microscope using a precalibrated ocular eye piece micrometer. Pixel Pro Software was used for particle size analysis. Mean particle sizes of all empty niosomes formulation and drug loaded niosomal formulations were determined by using optical microscopy (Labomed Microscope) having 10 X magnification and results were recorded [12].

***Determination Entrapment Efficiency Of Noisome (EE %)***

The unentraped drug was alienated from the niosomal dispersions by centrifugation at 15,000 rpm for 45 min. The supernatant was separated, diluted to 100 ml with PBS pH 7.4, filtered using a membrane filter (0.45µm pore size), and measured using UV (Schimadzu, UV 1700, Japan) spectrophotometer at 249nnm. EE% was calculated by the following equation [13, 14].

EE (%) - Ce /Ct X 100

EE (%) - % entrapment efficiency

Ce - concentration of entrapped drug

Ct - concentration of total drug

***Determination Of In-Vitro Drug Release (%)***

In-vitro drug release study of prepared niosomes was performed by using vertical Franz diffusion cell apparatus. The cellophane membrane was used for this study and this membrane was sandwiched between donor and acceptor compartment. The donor compartment was filed with niosomal dispersion and the acceptor compartment was filled with diffusion media (phosphate buffer saline pH 7.4). The temperature of the media was maintained at 37 ± 0.5oC. The entire assembly was placed on magnetic stirrer at constant speed (200 rpm) 2 ml sample was withdrawn at specific time interval for 2.5 hrs and replaced with fresh diffusion medium to maintain the sink condition. The further dilutions of sample were prepared and the samples were measured spectrophotometrically at 259 nm.[15]

***Stability Studies***

The stability studies for best topiramate niosomes formulation was carried out as per ICH guides for 3 months [21]. From this study, All formulations were divided into three sets and were stored at 4 ± 2°C, 25 ± 2°C/ 60 % RH ± 5 % RH and 37 ± 2°C/ 65 % RH ± 5 % RH in humidity control The niosomes were sampled at regular intervals of time (0,1,2,3 months), tested for % entrapment efficiency.[16,17]

## V. Result and Discussion

***FTIR***

By correlating peaks of pure drug spectrum with formulation, it was found that the drug is compatible with formulation component. The FTIR spectra showed the prominent peaks of various groups present in the Topiramate. The infrared spectra of Topiramate exhibited transmittance peaks at NH stretching at 3207 cm-1, -CH stretching at 2663 cm-1, S-O functional group at 1455 cm-1, and –CH bending at 756 cm-1.These characteristic peaks of the drug are well presented in niosomal formulation indicating absence of any interactions between drug with excipients.

## Fig. no. 1,2

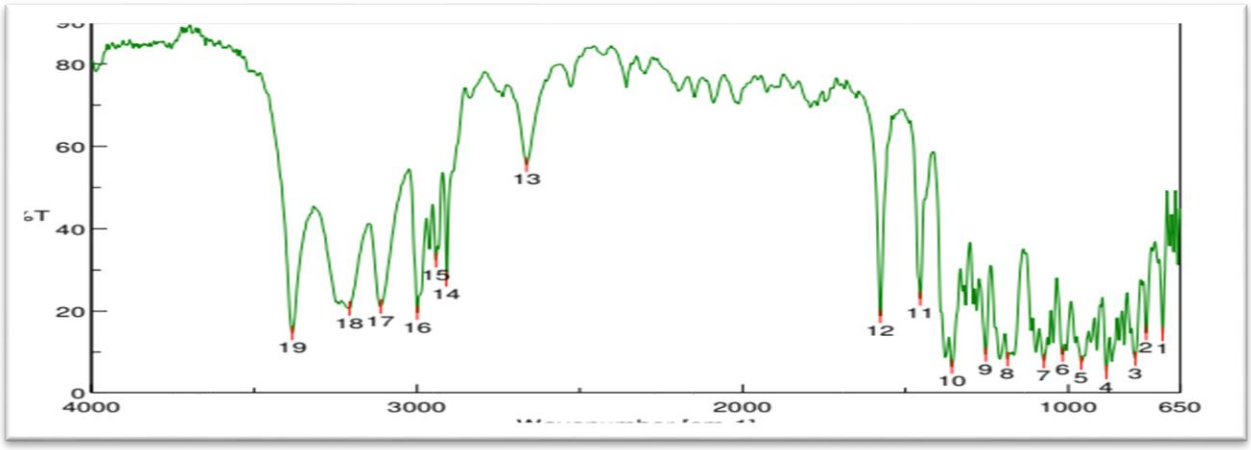


Fig1.: FTIR of Topiramate

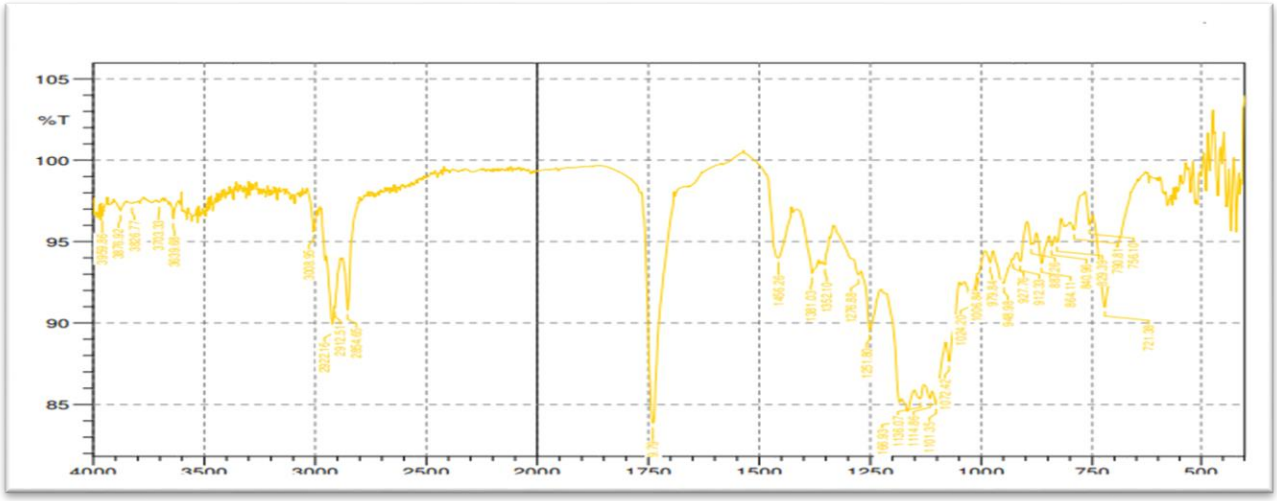


Fig2.: FTIR of Topiramate and Excipients

***DSC***

DSC thermogram of TPM was showed a indothermic peak at 124ºC corresponding to its reported melting point. On the other side shift in the thermogram of TPM niosomal dispersion was observed and peak was showed at 125ºC difference in the melting points of pure a TPM and niosomal dispersion of TPM was an indication of the increased solubility of TPM.

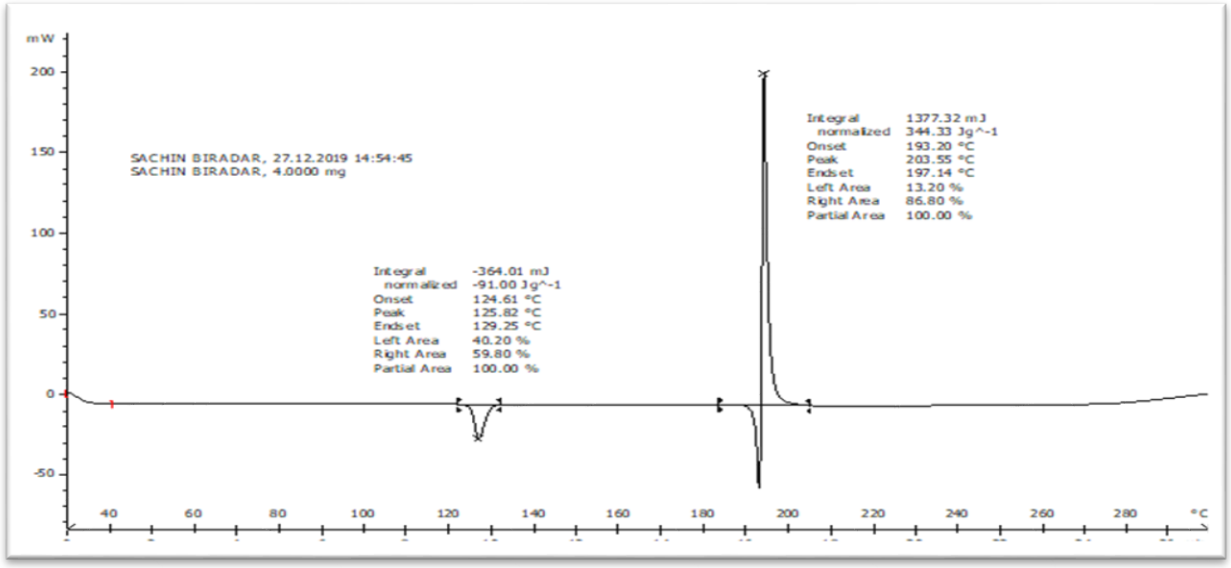


Fig3: DSC of Topiramate

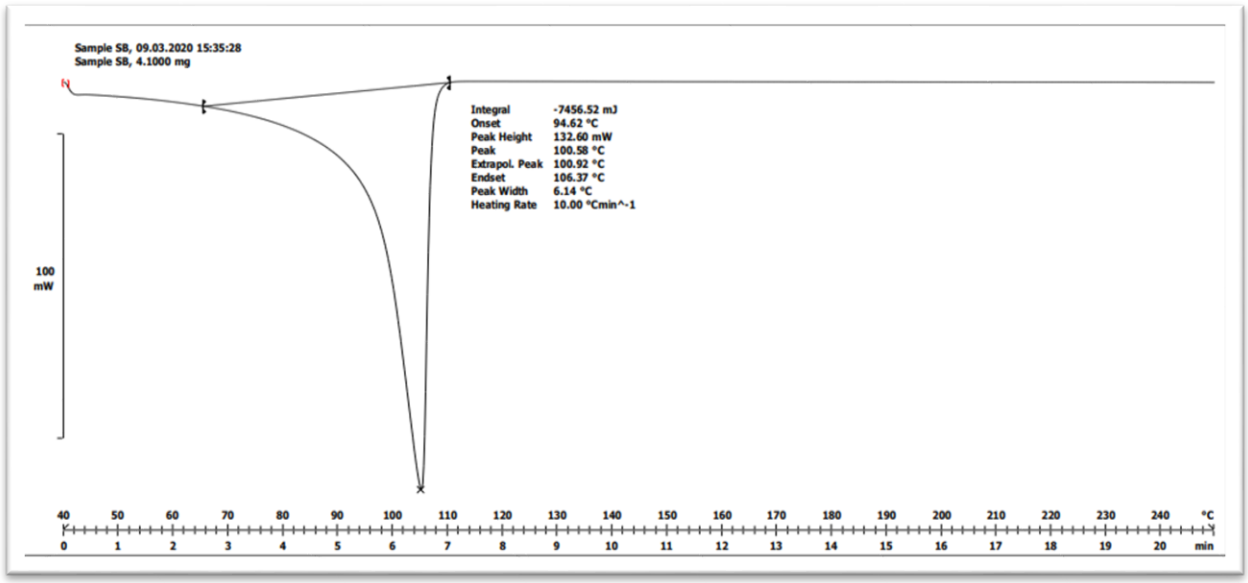


Fig4: DSC of Topiramate and Excipients

***Determination Of Vesicle Size***

P-values less than 0.0500 indicate model terms was found to be significant for vesicle size with model f value of 16.87(P-values less than 0.0500 ) in this case A, C, D, AB, AC, AD, BD, CD, $C^2D$ are significant model terms. The cubic equation generated by software is as fallows,

Vesicle size =+0.3432+0.0380 A+0.0006 B+0.0085 C-0.0118 D-0.0372 AB+0.0245 AC-0.0272 AD+0.0030 BC-0.0107 BD-0.0091 CD-0.0016 $A^2$-0.0003 $B^2$-0.0020 $C^2$-0.0017 ABD.

The equation in terms of coded factors can be inured to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is helpful for identifying the comparative impact of the factors by comparing the factor coefficients.

Above equation stated that all factors affect vesicle size significantly. 3D graph showed that as concentration of surfactant and cholesterol increases also vesicle size goes on increases. The vesicle size of all formulation were found to be in the range of 0.35nm

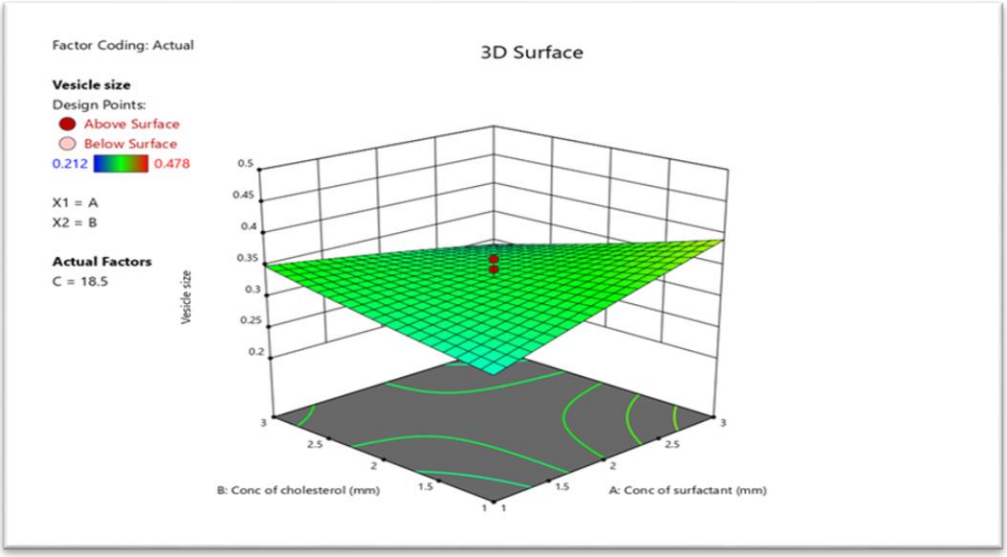


Fig no. 4. 3D graph for vesicle size

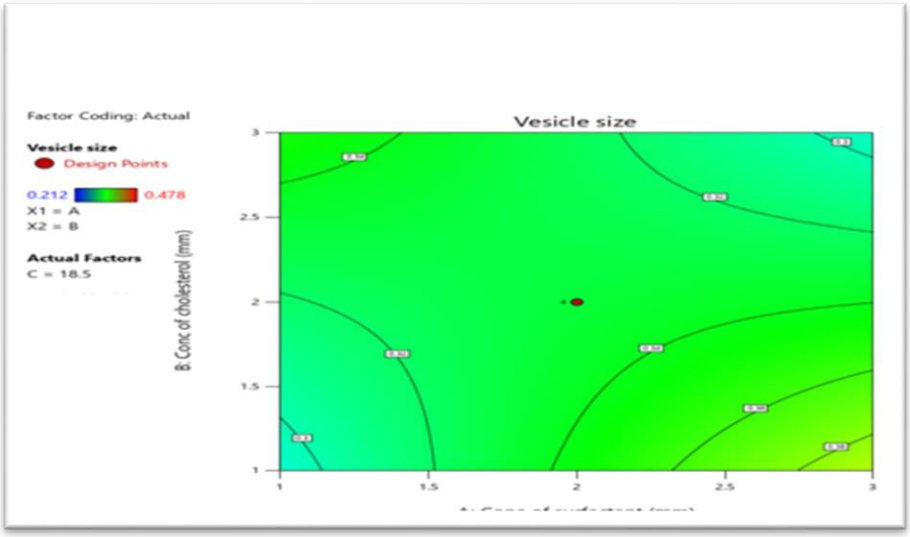


Fig no. 5 Contour plot for vesicle size

***Determination of Entrapment Efficiency***

P-values less than 0.0500 indicate model terms were found to be significant for % entrapment efficiency with model f value of 2.39 is significant. In this casing B, BD, CD, $B^2$ are significant model terms. The cubic equation generated by software is as fallows,

% entrapment =+93.08+1.51 A+2.59 B+0.0561 C-0.1602 D-0.3419 AB+0.6369 AC-0.1480 AD-1.19 BC-3.39 BD-2.42 CD-0.2928 $A^2$-1.91 $B^2$-1.01 $C^2$+1.89 ABD-1.36 ACD+0.4106 BCD-0.4307 $A^2D$+0.2994 $B^2D$.

The equation in terms of coded factors can be used to build predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is helpful for identifying the comparative impact of the factors by comparing the factor coefficients.

Above equation stated that all factors affect% entrapment efficiency significantly. 3D graph showed that as concentration of surfactant and cholesterol increases also % entrapment efficiency goes on increases. The % entrapment efficiency of all formulation were found to be in the range of 93.89%

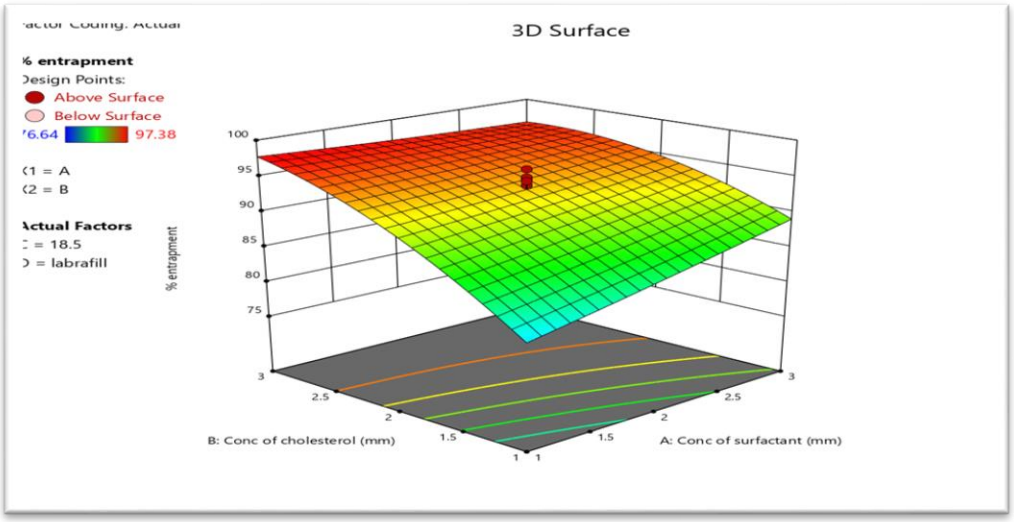


Fig no.6. 3D graph for % entrapment efficiency

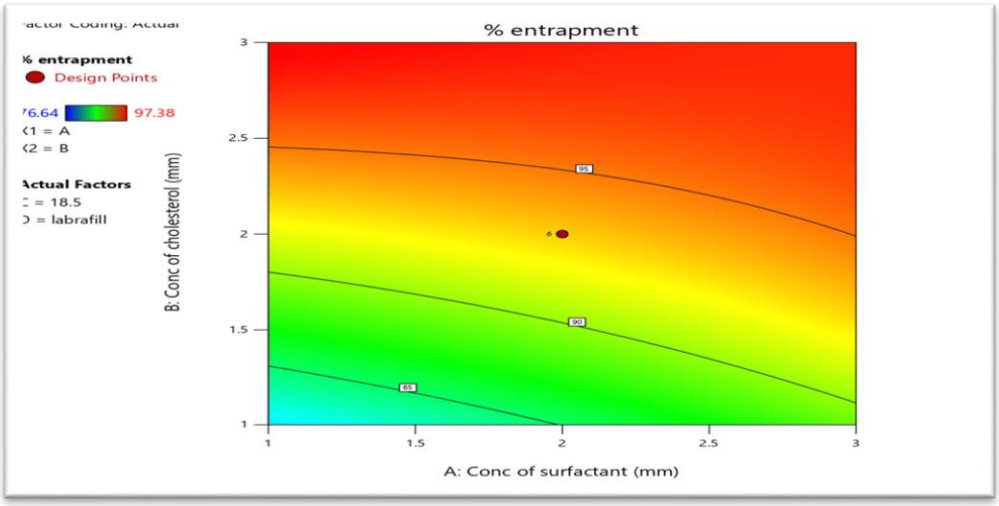


Fig. no. 7.Contour plot for % entrapment efficiency

***In-Vitro Drug Release Study***

P-values less than 0.0500 indicate model terms were found to be significant in-vitro drug release with model f value of 3.04 is significant. In this case A, B is significant model terms. The cubic equation generated by software is as fallows,

Drug release = +90.83-1.57 A-3.40 B-0.5066 C+1.40 D+1.60 AB-0.5794 AC-0.6269 AD-0.3069 BC-0.5011 BD+0.7662 CD+0.9132 $A^2$+0.2087 $B^2$-0.1625 $C^2$-0.7681 ABD+0.7881 AC-0.0844 BCD-0.4548 $A^2D$-0.3355 $B^2D$-1.03 $C^2D$.

The equation in conditions of coded factors can be used to make predictions about the reply for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is helpful for identifying the comparative impact of the factors by comparing the factor coefficients.

Above equation stated that all factors affect % drug release significantly. 3D graph showed that as concentration of surfactant and cholesterol increases also % drug release goes on increases. The % drug release of all formulation were found to be in the range of 80.94-92.74%. The % drug release of optimized formulation wad found to be 94-92.74% at 2:30 mins.

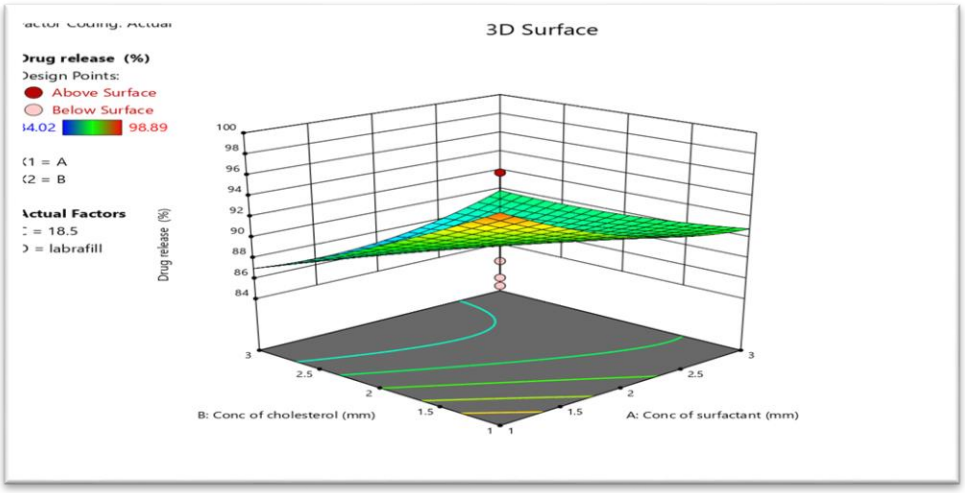


Fig no.8. 3D graph for In-vitro drug release

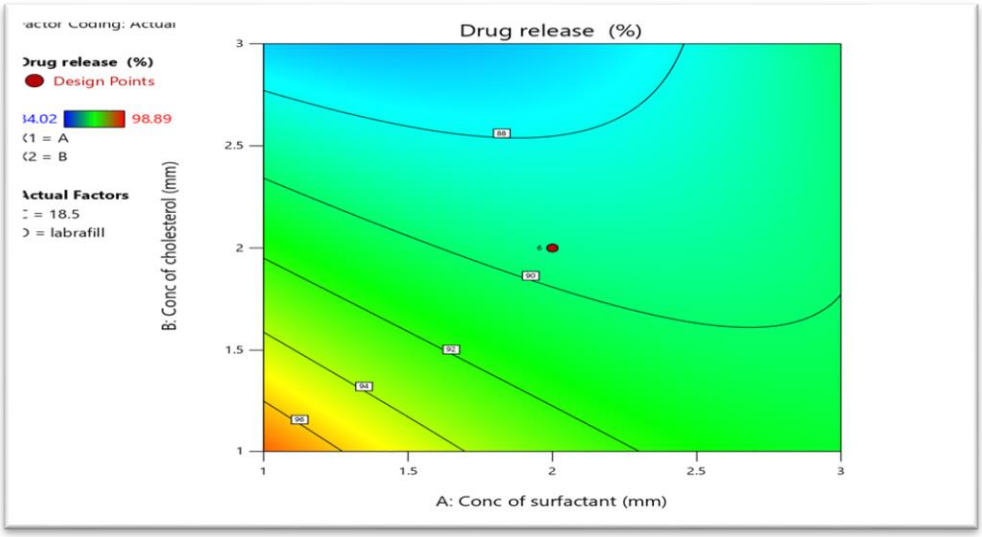


Fig. no.9 Contour plot for In-vitro drug release

***In-Vitro Drug Release of Topiramate***

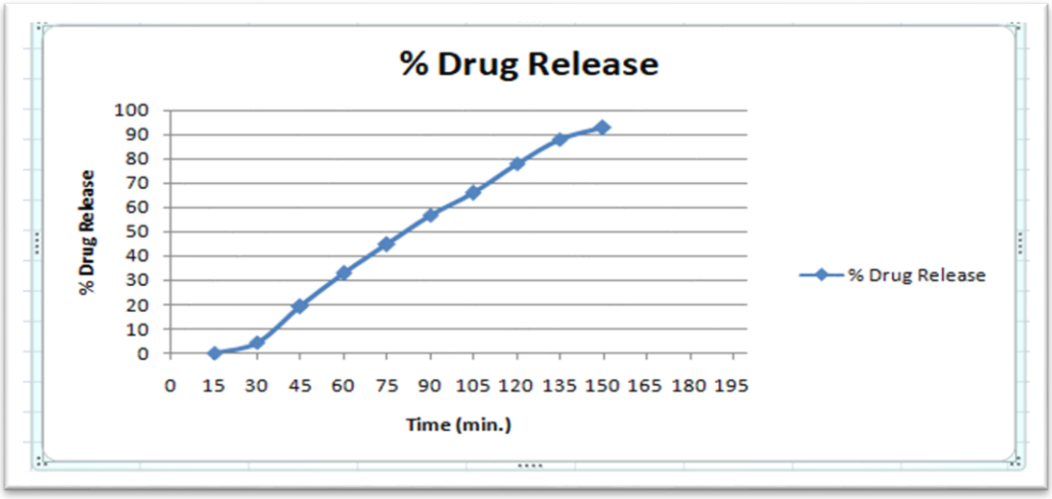


Fig no. 10: In-vitro drug release of Topiramate Niosome

## VI. Desirability and Optimized Batch

Design Expert Software measure being one having the maximum desirability value. The optimization process was performed by setting $Y_1$ at minimum, $Y_2$, $Y_3$, at maximum while all independent variables within range obtained. The optimized formulation was achieved at A= 2, B= 2, C= 18.5, D= Labrafil with corresponding desirability value of 0.809 (fig.5). This factor level combination predicted the responses $Y_1$=0.35µm, $Y_2$=93.24%, $Y_3$=89.42% where, observed responses was $Y_1$= 0.35 µm, $Y_2$=93.89%, $Y_3$=92.74%.

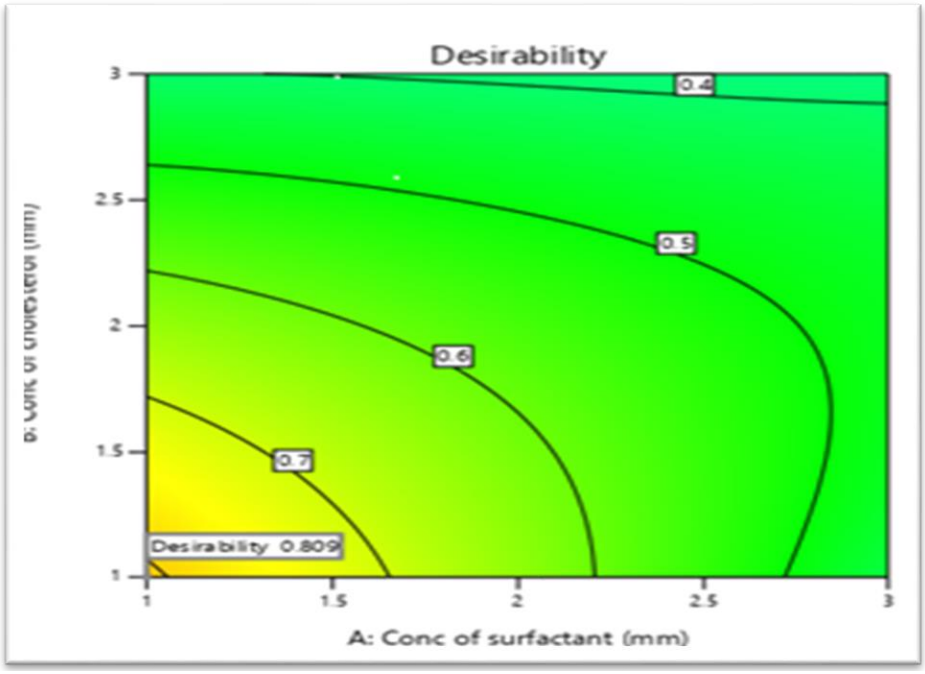


Fig no. 11: Desirability plot of Niosome

## VII. Stability Studies

Stability studies performed for optimized formulation of both niosomal Formulation. Formulation was stored at $4\pm2^0$C for a period of 30 days. Results were showed in Table.

| Sr. No. | Parameters | Initial days | After 30 days |
|---|---|---|---|
| 1 | Particle size | 0.35µm | 0.37µm |
| 2 | % Entrapment Efficiency of Topiramate | 94.64% | 94.62% |
| 3 | % Drug Release of Topiramate | 92.02% | 92.04% |

Table : Stability studies of Niosomal formulation

## VIII. Conclusion

The present study was aimed to develop and evaluate the niosomes of topiramate by using different surfactants Tween and Labrafil range in different concentration and keeping the cholesterol content constant. The highest entrapment was found to in Labrafil. Thus, it can be concluded that niosomes represents a promising drug delivery system.

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