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DEVELOPMENT OF SAFRANAL NIOSOMAL IN-SITU NASAL GEL FORMULATION

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ABSTRACT

The purpose of the present investigation was to prepare and evaluate formulations of safranal to improve solubility, stability and facilitate the blood brain barrier crossing of the drug. Niosomes are prepared using various surfactants in different ratios with cholesterol, and optimized based on vesicle size, drug entrapment, drug release and effect of sonication. Then to increase resident time of formulation in the nasal cavity optimized niosomal formulation (B1) further formulated in to in-situ nasal gel using surface response factorial Method. Concentration of Pluronic F-127 and gelrite were used independent variables as the polymers exhibited the phase transition behaviour and gelation temperature and gelation pH was used as dependent variable. The optimised gel concentration (pluronic F127: gelrite,(17.3:0.07) was then used to formulate the in situ niosomal nasal gel The prepared formulation was then characterized for drug loading, content uniformity, in-vitro drug diffusion, mucoadhesive strength, gel strength, viscosity and gelation temperature. The formulation so prepared was in the liquid state at room temperature while turned into a gel at the pH and temperature of the nasal cavity. Formulation showed the increased bioavailability of drug in in-vivo experiments.

KEYWORDS: safranal; entrapment efficacy; niosomes; formulation; stability

1. INTRODUCTION

Safranal is of natural origin having low solubility, low stability, low bioavailabity and facilitates faster crossing of blood brain barrier. So to overcome all these issues related to drug safranal is formulated in to niosomal formulation. Niosomes entraps the drug inside the

vesicle and thus increases its stability. Drug delivery system using colloidal particulate carrier such as liposomes [1] or niosomes [2] have distinct advantages over conventional dosage forms because the vesicles can act as a drug containing reservoirs. Niosomes are non-ionic surfactant based vehicles, biodegradable, biocompatible, non-immunogenic in nature, posses flexibility in their structured characterization,[3] and exhibit needlessness of handling or storing of niosomes in special conditions.[2, 4] These are developed as controlled drug delivery systems may be unilamellar or multilamellar depending on the method used to prepare them and they have been extensively studied for their potential to serve as carriers for delivery for drugs, antigens, hormone and other biogenetic agents.

Safranal is a monoterpene aldehyde,[7] formed in saffron by hydrolysis from picrocrocin during drying and storage. It is the main essential volatile oil responsible for the saffron characteristic such as odour. The average safranal content made up of 60% of the volatile fraction of the saffron.[8] Saffron is used in traditional medicine for treatment of various disorders[9,10] and safranal may at least in part be responsible for the therapeutic effects of the plant. Several different pharmacological effects for safranal have been demonstrated including: anxiolytic and hypnotic activity. [11-15] Safranal also has antioxidant properties. [16-20] The anticancer effect of Safranal is also demonstrated. [21,22] The antitussive activity of Crocus sativus stigma and petal extracts and its components, safranal and crocin, has been shown.[23] The relaxant effects of aqueous-ethanolic extracts of C. sativus and safranal[24] and their stimulatory effect on β-adrenoceptors were also demonstrated.[25] This study firstly focuses on formulation of stable safranal niosomes to increase solubility and bioavailability of safranal secondly on ease with which they can be administered to the patient by formulating as in situ nasal niosomal gel. and thirdly on the anticonvulsant and antidepressant activity of safranal.

2. MATERIALS AND METHODS

2.1 Materials

Safranal is purchased from Sigma Aldrich. Polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate (Span 80), Methanol and Chloroform were purchased from S.D.Fine Chemicals, Mumbai. Cholesterol was purchased from Loba Chemi, Mumbai. Polymer Pluronic F-127, Glucire 44/50 was purchased from Sigma Aldrich (USA).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.

2.2 Method of Preparation of Niosome

Niosomes containing safranal were prepared by ether injection technique using non-ionic surfactants Span80 and Tween 20 & 80, gelucire 44/50, pluronic f-127 and cholesterol at different ratios. Cholesterol and surfactants were dissolved in 6ml diethyl ether mixed with 2ml methanol containing weighed quantity of safranal. The obtained solution was slowly injected by using micro syringe, at a rate of 1ml/min via 14-gauge needle into 15ml of aqueous phase maintained at temperature 60^0C. The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60-65 ºC. Different batches of niosomes were prepared in order to select an optimized formula as per the general method described above. The proportion of surfactants and the fixed proportion of cholesterol for the preparations of niosomes given in Table 1.

Table 1 Formulations of Niosomes

Sr. No.	Batch	Chol: Surfactant
1	span 80 (A1-A6)/ tween 80 (B1-B6)/ tween 20(C1-C6)/ pluronic f127 (D1-D6) gelucire (E1-E6) (amount of drug incorporated 25mg)	1:1.5
2		1:2.5
3		1:3.0
4		1:3.5
5		1:4.5
6		1:6.0

2.3 Evaluations of niosomes

2.3.1. Vesicle diameter

The size, shape, and lamellar nature of vesicles in sonicated formulations were observed by optical microscopy (Kandasamy Ruckmani et.al 2010) using a calibrated eyepiece micrometer and photographs were taken at x400 magnification with a digital camera (Motic, 8.1 megapixel, Japan).

2.3.2. Determination of drug entrapment in vesicles

Safranal niosomal formulations were centrifuged at 15000 rpm for 90 min at 4^0C using a refrigerated centrifuge (Eppendorf, 5415 R, Germany) to separate niosomes from non-entrapped drug. Concentration of the free drug in the supernatant was determined by measuring absorbance at 308 nm with a UV spectrophotometer (Shimadzu, UV 1650 PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated. This process was repeated thrice to ensure that free drug was completely removed.

%drug entrapment =[(total drug -drug in supernatant)/ total drug] 100

Percent drug entrapment was confirmed by lysing the niosomes with n-propanol after centrifugation and measuring absorbance at 308 nm.

2.3.3 Determination of viscosity

Viscosity of the formulations was determined using an Ostwald viscometer at room temperature.

2.3.4. Drug compatibility study

FTIR spectra of safranal (drug), excipient and optimized formulation were recorded on Shimadzu FTIR 8400 spectrophotometer. Sample was placed in sample holder, the scanning was performed between 4000 cm^{-1} to 400 cm^{-1} range.

2.3.5. Zeta potential determination

Vesicle size distribution, zeta potential and polydispersity index of the safranal niosome was determined using Zetasizer (model: nano zs, malvern instrument, westborough, MA,USA).

2.3.6. Effect of sonication on the vesicle size of the niosome

For evaluating the effect of Sonication on the vesicle size of the niosome two sets of niosomal formulation are prepared. First set of niosomal formulation is sonicated during formulation and other set is kept unsonicated and observations are carried out.

2.3.7 *In vitro* release of safranal from niosomes

The release of safranal from niosomes was determined using the membrane diffusion technique; solution was constantly stirred at speed 50 rpm at 37 ±1 ^{0}C on a magnetic stirrer with a thermostat. Aliquots were withdrawn at specific intervals and replaced simultaneously with equal volume of fresh PBS. The safranal concentration in the samples was analyzed

spectrophotometrically, as mentioned earlier. The obtained data were analyzed to determine the amount and mechanism of drug release.

2.4 Formulation of niosomal in-situ nasal gel

Aqueous gels containing admix of PF-127 and gelrite were prepared containing mixture of PF-127 and gelrite. Polymer slowly added to cold distilled water with continuous stirring. Mixture was kept overnight at 4^0C to ensure complete dissolution. After estimating the gelation temperature, the optimized concentration of PF-127 and gelrite was used for further niosomal gel formulation. Niosomal gel was prepared using the same formula and the resultant dispersion was stored at 4^0C in a refrigerator for further studies.

2.5 Optimization Of niosomal gel

The prepared gels by taking various ratios of gelling agents are evaluated for gelation temperature and gelation pH. Surface response Factorial design was constructed to estimate the best amount of surfactant in formulating safranal niosomal in situ nasal gel, with combinations from two factors (independent variables) which were concentration of gelling agents. The responses of model formulations were treated by Design-Expert® version 8.0.7.1 software.

Table 2 Variables in optimization study

Variables	Factor
Independent	
X1	Concentration of gelrite (0-0.5%)
X2	Concentration of Pluronic F 127 (0.01-18%)
Dependent	
Y1	Gelation Temp (^{0}C)
Y2	Gelation pH

Table 3 Formulations for optimization of nasal gel

F.C.	Conc. of Gelrite	Conc. of Pluronic F-127
G1	1.000	-1.000
G2	0.000	1.414
G3	1.414	0.000
G4	-1.000	1.000

G5	0.000	0.000
G6	0.000	0.000
G7	1.000	1.000
G8	-0.586	0.000
G9	0.000	0.000
G10	0.000	0.000
G11	1.000	-1.000
G12	0.000	-1.414
G13	0.000	0.000

2.6 Characterization of niosomal in situ nasal gel

The above formulated PF-127 and gelrite niosomal in-situ-nasal gels of Safranal were subjected to evaluation for visual appearance, clarity and pH, drug content analysis, the mucoadhesive forces, gel strength, in vitro drug diffusion studies, estimation of gelation and toxicity study.

2.6.1. Visual Appearance, clarity and pH

Visual appearance and clarity were observed for the presence of any particular matter. The pH of in-situ gels was measured using digital pH meter.

2.6.2. Drug content analysis

It was carried out using UV-Spectrometric method and sufficient amount of methanol was added to lyses the vesicles. Then 0.1ml of formulation was diluted to 100ml of simulated nasal fluid pH7.4 and the absorbance was measured at 308nm using simulated nasal fluid pH 7.4 as blank.

2.6.3. The mucoadhesive forces

The mucoadhesive forces of the gels were determined by means of modified analytical two-pan balance using nasal mucosa of goat. Weights were added at a constant rate to the pan on the other side of the modified balance of the used device until the gel gets detached from tissue. The mucoadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights required for the detachment using the following formula,

Detachment stress (dynes/cm2) = mg / A

Where, m= the weight added to the balance in gram.

g = acceleration due to gravity taken as 980 cm/sec^2.

A= area of tissue exposed.

(The surface area of mucosa was 1.12cm^2)

2.6.4. Gel strength

Formulated gels were placed in the test tubes and gelled in a thermostat at 37 ^{0}C. The apparatus for measuring gel strength (Weight: 27gm.) was then placed onto the niosomal gel formulation. The time taken by the apparatus to sink to a depth of 5 cm through the prepared gel was measured for Tween niosomal gel formulations.

2.6.5. *In vitro* drug diffusion studies

The horizontal diffusion chamber was used for the present study using goat nasal mucosa. Phosphate buffer solution of pH 7.4 was used in the receptor chamber. Before starting the study, the mucosa was pre-incubated with phosphate buffer solution of pH 7.4 so as to saturate the mucosa; so that there should not be any change in permeability. The niosomal nasal gel formulation solution was taken into the donor compartment (quantity of the niosomal gel formulation was approximately about 1-1.5 ml of gel). The speed of the magnet was adjusted at an optimum speed. Sampling was done at regular intervals, i.e. for 15, 30, 45, 60, 75, 90, 120, 180, 240 and 300 min. The sink condition was maintained with phosphate buffer solution. The samples were diluted with methanol and further measurements were carried out on the UV spectrophotometer at 308 nm.

2.6.6. Gelation study

The gelation temperature was measured by heating the gel formulation (about 1-2^{0}C) in a test tube with gentle stirring till the gel is formed. Gelation was considered at the point where there was no flow seen when the test tube was overturned.

The gel-melting temperature was recorded at a point when the gel starts flowing upon tilting the tube through an angle of 90^0.

2.6.7. Toxicity study

The toxicity study was carried out by adding the formulation to the fresh blood sample and keeping it under observation along with fresh blood sample without formulation.

2.6.8 In vivo study

In vivo study was carried out for two activities,

Firstly, anticonvulsant activity in mice was evaluated using the strychnine as an convulsion inducer. In this test, strychnine (4 mg/kg, s.c.) was injected to the animal 15 minutes post administration of safranal loaded niosome administration by intravenous route. Animals were observed for 10 min for onset on various seizures latencies were noted in seconds. If any seizure type was not observed up to 10 min. then the latency of 600 sec is assigned as cut-off latency. Abolition of the hind limb tonic extensor component was taken as the endpoint

Secondly, antidepressant activity in mice was evaluated by swim test, swim time of the mice was recorded for controlled and test group.

3. RESULTS AND DISCUSSION

3.1. Characterization of niosomes

3.1.1. Vesicle diameter

Vesicle diameter for tween 80, span 80, tween 20 and pluronic F-127 was found to be in range of 0.6±0.1µm where for gelucire the vesicle diameter was more than 1µm

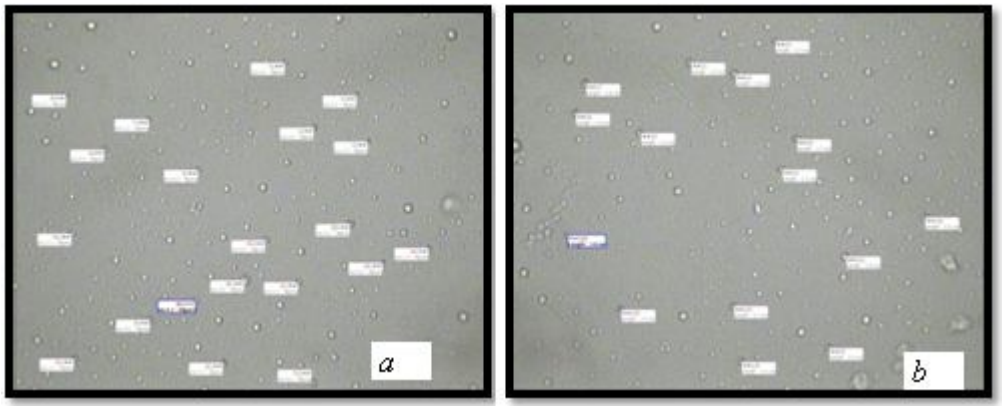

Figure 1 Photomicrograph of safranal loaded niosomes composed of tween 80 and cholesterol (a) chol.: tween 80(7.6:39.3) (b) chol.: tween 80(7.6:65.5)

3.1.2. Entrapment efficiency

The effect of Different concentration of tweens (20, 80), span 80, pluronic F-127, gelucire on the entrapment is depicted in Table 4. Entrapment efficiency for niosome prepared with tween 80 was higher indicating lower the HLB higher the entrapment. The entrapment efficiency of span was more than tweens, pluronic and gelucire might be due to lower HLB.

3.1.3. *In vitro* release

As tween 80 possesses one saturated C=C in the alkyl moiety of the hydrophobic substrate nearly lies parallel to the force of unsaturated molecule in tween 80 molecule which might

have increased percentage release for tween 80 niosomes. The drug release was found to be in the decreasing order as tween 80 > tween 20 > span 80 > pluronic f-127 > gelucire.

3.1.4. Viscosity

Viscosity for niosomes was high for tweens and was 1.11 ±0.014 to 1.23 ±0.043 poise than other surfactant 1.16 ±0.07-1.58 ±0.4 poise and it might be due to vesicle size variation of the niosomes (table2).

Table 4 Mean vesicle size, viscosity, % entrapment efficiency and in vitro release of prepared niosomal formulation

Sample ID	Vesicle Size (µm)	% Entrapment	% Release	Viscosity (poise)
A-1	0.6 ±0.049	98.94±1.04	78.22±0.4	1.23±0.028
A -2	0.57±0.007	98.85±.059	72.39±0.74	1.32±0.028
A-3	0.64±0.028	98.83±0.60	67.28±0.91	1.44±0.014
A-4	0.96±0.098	98.72±1.3	65.86±1.3	1.35±0.007
A-5	0.78±0.028	98.79±1.03	62.67±1.01	1.39±0.02
A-6	0.67±0.056	98.64±1.4	68.76±0.91	1.34±0.28
B-1	0.58±0.014	98.55±.93	90.44±0.93	1.18±0.049
B-2	0.65 ±0.035	98.43±1.6	85.66±0.98	1.23±0.042
B-3	0.6±0.056	98.3±1.3	83.63±1.06	1.22±0.091
B-4	0.75±0.014	98.19±1.66	75.94±1.47	1.11±0.014
B-5	0.73±0.049	98.38±1.48	71.98±1.48	1.18±0.056
B-6	0.71±0.042	98.28±.96	68.76±1.32	1.16±0.49
C-1	0.58±0.021	97.96±1.5	78.75±1.44	1.52±0.07
C-2	0.56±0.035	98.12±.89	77.14±0.95	1.44±0.056
C-3	0.6±0.077	97.63±2.2	74.62±1.51	1.31±0.04
C-4	0.78±0.098	97.49±1.4	72.49±1.27	1.22±0.021
C-5	0.68±0.070	97.77±1.7	71.21±.96	1.19±0.091
C-6	0.64±0.063	97.56±0.5	68.47±1.4	1.28±0.084
D-1	0.67±0.056	89.7±1.64	76.01±.54	1.25±0.063
D-2	0.65±0.056	88.3±2.17	69.97±1.4	1.28±0.056

D-3	0.58±0.028	88±2.03	68.32±0.58	1.32±0.007
D-4	0.77±0.035	87.2±.92	66.27±0.98	1.25±0.091
D-5	0.83±0.07	89.5±1.18	64.98±1.24	1.29±0.056
D-6	0.72±0.16	88.1±1.01	65.44±1.24	1.33±0.106
E-1	0.99±0.14	96.68±1.5	65.71±1.08	1.23±0.014
E-2	1.05±0.084	96.32±.98	68.09±0.5	1.2±0.077
E-3	1.08±0.021	96.26±1.6	64.41±1.35	1.36±0.098
E-4	1.01±0.028	95.79±1.1	59.89±1.2	1.37±0.014
E-5	1.09±0.049	95.25±1.2	57.84±0.58	1.22±0.007
E-6	1.14±0.028	96.09±.1	52.45±0.39	1.38±0.091

Based on the drug release niosome containing tween 80 as a surfactant i.e. formulation B1 was optimized and selected for the further study.

3.1.5. Drug compatibility study by FTIR

The pure drug safranal exhibit characteristic peak at (C=C) stretching at 1452 cm^{-1}, (C=O) stretching at 1715 $cm^{-1,}$ (C-H) stretching at 3200-2800 cm^{-1}. In tween 80 ketonic functional group gives peak at 1651 cm^{-1} and (-OH) bending at 1058 cm^{-1}. No additional absorption bands were observed in the optimized formulation. From the result it clearly understood the spectra of optimized formulation were found to be identical with that of pure drug.

Thus it was concluded that, there was Drug was enclosed in the vesicles and therefore did not show the peak between the drug and excipient, hence the drug and excipient were compatible and stable, shown in fig.2

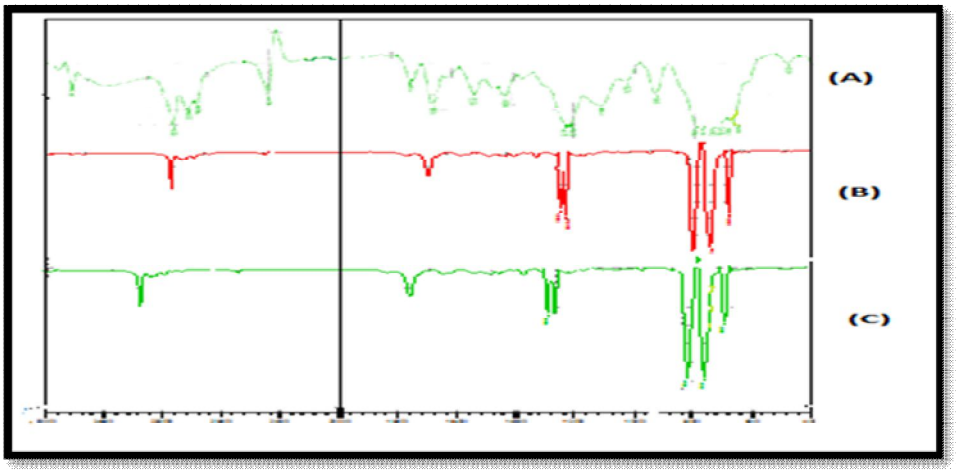

Figure 2 FT-IR spectra of (A) Safranal, (B) Tween 80, (C) Optimised formulation (B1)

3.1.6. Zeta potential, vesicle size distribution and polydispersity index determination

Zeta potential, vesicle size distribution and polydispersity index of optimised niosomal formulation was determined. It was clear from the observation that as the surfactant changes the zeta potential and polydispersity index of the formulation also changes. Zeta potential is greater for tween formulations (17-30 mV) than span (-23 to 20 mV), pluronic f-127 (11-18mV) and gelucire (17-24 mV) formulations and polydispersity value is in the range of (0.4-0.9) for all the formulation had nearly similar vesicle size distribution as shown in fig.3

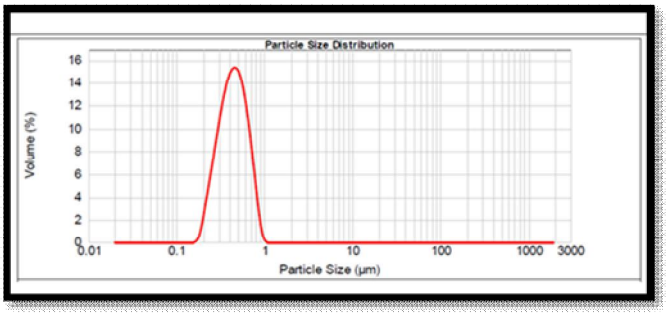

Figure 3 Vesicle size distribution of niosomal formulation

3.1.7. Effect of sonication on the vesicle diameter of niosome

Effect of sonication on the vesicle diameter of niosome is observed by determining the vesicle diameter of sonicated and unsonicated niosomal formulation. There is remarkable difference in vesicle diameter of both the formulation the unsonicated formulations have larger vesicle diameter(average vesicle size is1.38µm) as compared with the sonicated one (average vesicle size is 0.65µm) (fig.4).

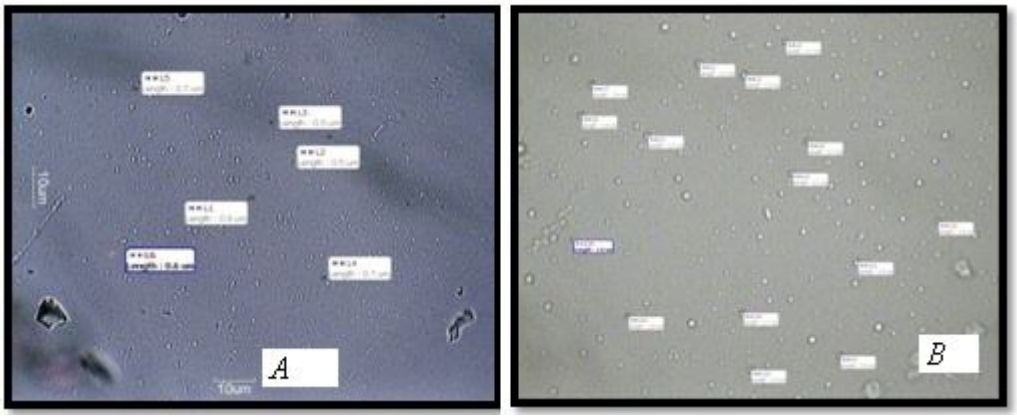

Figure 4 Vesicle size distribution of sonicated niosomes(A) and unsonicated niosomes (B)

3.2. Optimization of niosomal gel

Table 5 Summary of results of regression analysis for response

Response	Models	F value	Prob > F	R^2	Adjusted R^2	Predicted R^2	S.D.	Remarks
Y_1 (gelation temp.)	2FI	455.1	< 0.0001	0.9935	0.9913	0.9753	1.63	Suggested
Y2 (gelation pH)	Quadratic Model	186.5	< 0.0001	0.9926	0.9872	0.9034	0.28	Suggested

Equation: $Y = b_0 + b_1X_1 + b_2X_2 + b_1^2X_1X_2 + b_1^2X_1^2 + b_2^2X_2^2$

gelation temp

$Y1 = 45.51 - 14.88\ X_1 - 13.57\ X_2 + 13.88\ X_1X_2$

gelation pH

$Y2 = 5.42 - 2.85\ X_1 - 1.14\ X_2 + 1.78\ X_1X_2 + 2.26\ X_1^2 - 0.18\ X_2^2$

To optimize the concentration of Pluronic F-127 and gelrite for gelling purpose, the gelation study was done by taking various concentrations of PF-127 and gelrite. The concentration of 17.3% of pluronic F127 an 0.07% of gelrite was finalise as it showed the gelation at temperature of 34.5^{0}C and pH 6.3 which is the temperature and pH of Nasal cavity and nasal secretions.

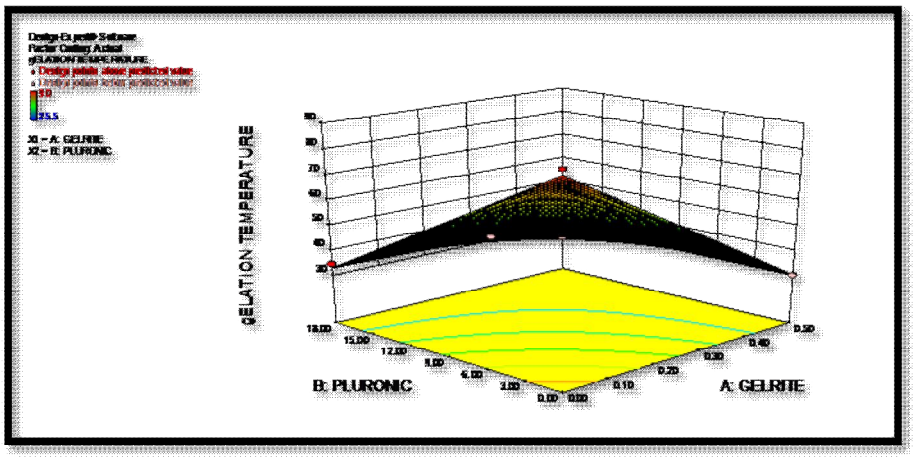

Figure 5 Response surface plot for gelation temperature

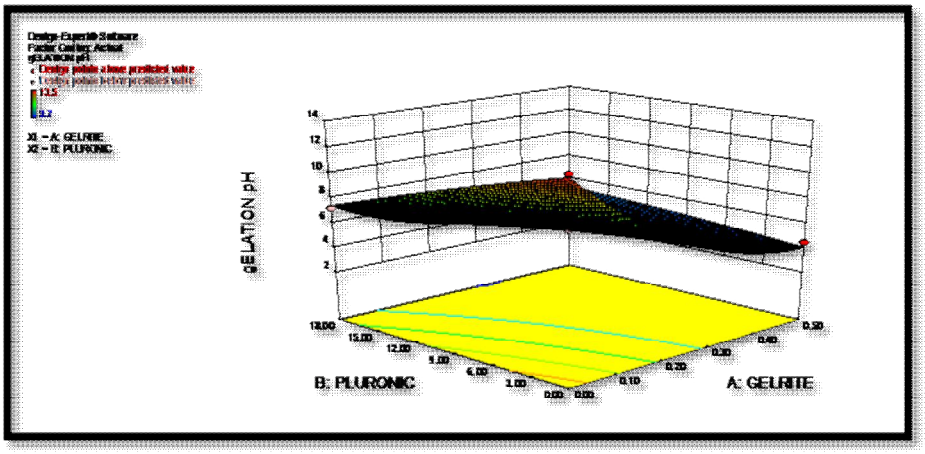

Figure 6 Response surface plot for gelation pH

From the above graph it is concluded that with the increase in pluronic f- 127 concentration gelation temperature decreases and with the increase in gelrite concentration gelation pH decreases.

The statistically significant relationship between the dependent and independent variables are Constructed based on the ANOVA results (Table 6). The effect of conc. of gelrite (X1) and concentration of pluronic (X2) gelation temperature (Y1) and gelation pH (Y2) is considered as response Fig. 5. Change in concentration of surfactants would leads to change in gelation temperature. Increasing the concentration of pluronic f-127 eventually decreases the gelation temperature and increase in gelrite concentration decrease the gelation pH. Gelation temperature looks independent of gelrite concentration as it not affect the response. From the obtained results it can be concluded that an optimal gelling concentration of gels for formulating safranal loaded niosomal in situ nasal gel formulation may be composed of Pluronic F 127(17.3%) and gelrite(0.07%) as a surfactant.

As the temperature of nasal cavity is 34^0C, our aim was to formulate the preparation that may gel at this temperature. If the gelation is below 34^0C or at room temperature it may lead to difficulty in manufacturing, handling and administration similarly if the temperature is above 34^0C than it would lead to rapid clearance of the drug from nasal cavity. Keck T et.al., suggested mechanism of gelation due to progressive dehydration of the polymer micelles of polyoxypropylene chains as temperature increase leading to increased chain entanglement due to conformational changes in the orientation of methyl groups in the side chains. Gelrite is an ion sensitive polymer which forms clear gel on contact with monovalent as well as divalent cations (Ca^{+2}, Na^{+}, K^{+}). In an ion free medium it forms double helices which at

normal condition are only weakly attached to each other (by Vander waals forces). In the presence of cations some of the helices associated in to aggregates and cause cross linking of polymer chains. The divalent ions viz, magnesium and calcium are superior to the cations sodium or potassium in promoting the gelation of polysaccharide. In the presence of soluble salts, gelrite can be used to provide high gel strength at low gelrite concentrations. These temperatures were found to be optimum for in-situ gelling of formulation with minimum loss of administered dose by clearance from the site of application. Gelation studies on mixture of PF-127 and gelrite gels and in presence of tween 80 formulations showed that with increase in concentration the gelation temperature decreases. This might be due to higher number and volume occupied by micelles at lower temperature.

- **Enthalpy Study**

At higher temperatures disruption in miceller arrangement occurs which are indicated by negative value of enthalpy of gel melting. The enthalpy of gelation depends on the type and extent of interaction occurred like its solubility in water. As such not much effect is seen on enthalpy of gelation and gel melting. A negligible difference of 0.5Kcal/mol increase in gel melting and 0.02Kcal/mol increase in gelation is seen which implies that there is no interaction between the niosomal formulation and the gel when incorporated in the system. Mucoadhesive strength was found for two minutes of contact time as per the study carried out by Rita J.Majithiya et.al, 2006. Analysis of mucoadhesive strength for PF127+ gelrite and other gel formulations showed that the detachment stress was more. Out of all the gel formulations that is containing tween niosomes in gel showed highest mucoadhesive force and gel strength indicating a significant improvement in the drug residence time.

Table 6 Enthalpies of gelation and gel melting.

Formulations	ΔH gel (kcal/mol)	ΔH melting (kcal/mol
Blank	1.751321±0.254	-3.613112±0.129
With niosome	1.832543±0.221	-3.654322±0.118

3.3 *In vitro* drug diffusion studies

The horizontal diffusion chamber was used for the present study using goat nasal mucosa. Phosphate buffer solution of pH7.4 was used in the receptor chamber. Before starting the study, the mucosa was pre-incubated with phosphate buffer solution of pH 7.4 so as to

saturate the mucosa; so that there should not be any change in permeability. The niosomal nasal gel formulation solution was taken into the donor compartment. The quantity of the niosomal gel formulation was approximately about 1-1.5 ml of gel. The speed of the magnet was adjusted at an optimum speed. Sampling was done at regular intervals, i.e. for 15, 30, 45, 60, 75, 90, 120, 180, 210,240 and 300 min. The sink condition was maintained with phosphate buffer solution. The samples were diluted with methanol and further measurements were carried out on the UV spectrophotometer at 308 nm.

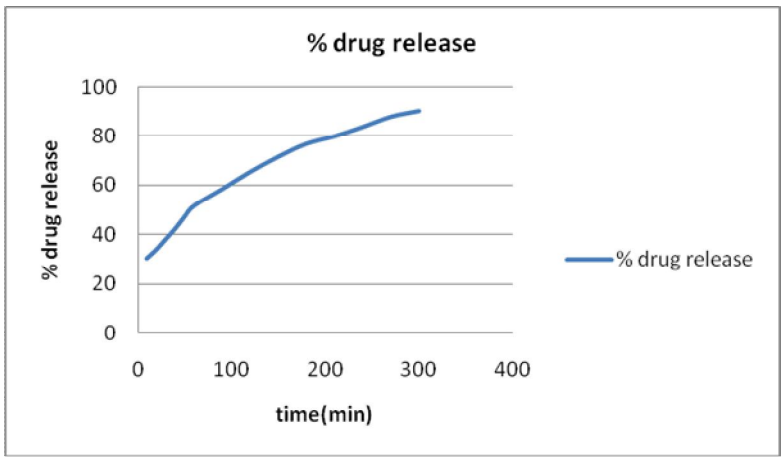

Figure 7 *In-vitro* release of drug for optimized safranal loaded niosomal in situ nasal gel.

3.4. Characterization of optimized niosomal gel formulations

Vesicle Size for all the formulation was in the range of 0.6-0.68 µm, Viscosity varies with varying the surfactant concentration, the gel containing 17.3% of PF-127 and 0.08 % gelrite gave optimum viscosity required for in situ phenomena and above that concentration viscosity increases above the level and below that concentration formulation did not form in situ gel. Mucoadhesive Strength for PF-127 and gelrite in concentration of 17.31 % and 0.07 % respectively possessed adhesive properties as well as gel strength increased with incorporation of tween niosomal formulation in it. Pluronic has both polyoxyethylene and polyoxypropylene groups it showed both hydrophilic and hydrophobic activity. With the increase of the length of the ethylene oxide chain (hydrophilic) of a polyoxyethylene non-ionic surfactant (Tweens) results in increase of surface tension. pH of the gel formulation was in the range of 6.22-6.85 and optimized gel has pH 6.63, Drug Content , Percentage release for the optimized niosomal gel was up to 90% where as for other was in the range of 88%-80%.

Table 7 Evaluation parameters of optimize niosomal gel formulation

Sr. No.	Parameter	Observation
1	Vesicle Size (μm)	0.65±0.014
2	Viscosity (Cp)	3150±0.4
3	Mucoadhesive Strength (Dyne/Cm2)	4292±0.6
4	Gel Strength (Sec)	97±0.5
5	pH	6.68±0.03
6	Drug Content	98.55±.93
7	Percent Release	90.44±0.93

Table 8 Effect of safranal loaded niosomal formulation on strychnine induced lethal seizure in mice

Group No.	Group Description	Onset time(sec)			
			Myoclonic seizures	**Clonic seizures**	**Tonic Extensor**
I	Control group	Mean	186.1	200.5	219.6
		S.D.	17.5	17.6	14.5
II	Safranal niosomal formulation (0.35 ml/kg I.V.)	Mean	445	472	492.5
		S.D.	25.3	20.7	25.2

3.5 Toxicity study

The toxicity study was carried out by adding the formulation to the fresh blood sample and keeping it under observation along with fresh blood sample without formulation. No excess concentration of Hb detected in blood sample with safranal formulation which shows that there will be no excess RBC breakdown due to formulation hence there will be no toxicity issue with the safranal niosomal gel formulation.

3.6 *In vivo* study

❖ **Anticonvulsant activity**

Strychnine induced seizures were characterized by tremors and straub tail followed by myoclonic jerks of the limbs, leading to full generalized clonic/tonic seizures. Generally, death occurred within few minutes of the onset of clonic/tonic seizures or, in any case, within

one hour. Vehicle failed to protect the mice from generalized clonic-tonic convulsions induced by strychnine. Safranal significantly delay the onset of myoclonic, clonic and tonic extensor compared to vehicle group ($p<0.05$).

❖ **Antidepressant activity:**

Antidepressant activity was characterized swim test, test group administered with safranal niosomes significantly enhanced the swimming time as compared with control group.

Table 9 Effect of safranal loaded niosomal formulation on swimming time.

Group No.	Group Description	Swimming Time (sec)	
I	Control group	Mean	72
		S.D.	±5.03
II	Safranal niosomal formulation	Mean	302
		S.D.	±7.16

4. CONCLUSION

The present study was an attempt to develop and evaluate the niosomal *in-situ* nasal formulations of Safranal by using different surfactants in different concentration and keeping the cholesterol content constant. The niosomes entrapped *in-situ* nasal gel formulations were able to release the sufficient quantity of drug in order to provide immediate relief from convulsion which was one of the objectives of the present study. The niosomal gel prepared with PF127 and gelrite was evaluated for gelation study and the other parameters like mucoadhesive strength and gel strength, and showed satisfactory results. Most importantly, the *in-vitro* release of the drug through the gel across the cellophane membrane was satisfactory and showed the controlled pattern release. There was drastic increase in the viscosity of formulation at the temperature of the nasal cavity indicating the occurrence of in-situ gelling phenomenon. Moreover, this formulation also provides the ease of administration as it is in the liquid form at non-physiologic conditions and thus helps in increasing patient compliance. Hence we can conclude that the niosomal *in-situ* nasal gel system can be considered as a promising approach for the anticonvulsive and anti depressive drug safranal.

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