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Niosomal *in situ* Nasal gel formulation of Doxepin HCL: A promising approach for Depression treatment

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Abstract

This study majorly focuses on the formulation of the Doxepin HCL filled niosomal in-situ nasal gel for depression therapy. Doxepin HCL in situ nasal gel was created with the Ether injection method. The formulation was modified to increase the bioavailability of the drug molecules. There are two barriers present in brain which are known as CSF barrier and BBB that produces hinderance to molecules to transport to the brain, but they allow lipophilic drug molecules to permeate. The particle size of optimized batch was found to be 422.75±110.65 nm. The entrapment efficiency of optimised formulation was found to be 88.89%. The niosomes were optimized by 2^3 factorials. And optimized batch of niosomes is incorporated into gel in 1:1 ratio. After eight hours of in-vitro release, the highest release was discovered to be 80.12%.

Key words : Niosomes, Antidepressant, Doxepin HCL, In-Situ nasal gel, Viscosity, Ether injection.

Depression is a condition in which mood level is very low and it affects the persons thought process, creativity, decision making power, feelings, etc. Depression comes under major psychotic disorder and it is mostly identified by unable to focus on one thing and suicidal thoughts.

Around 16% of world population is suffering from depression and which is primary cause of suicide in 60% of instances[17]. Despite the facts that depression is not an incurable disease, suicides have been reported in worst case situations[3]. Many of the patients doesn't responds to many of the antidepressant drugs

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which are present in the market[21] One of the major reasons behind failure of the major of the antidepressant drug is Blood Brain Barrier (BBB) which is the main reason for the hindrance of transport of drug to the CNS. The primary location of blood–CNS exchange is the blood–brain barrier (BBB), which is mostly made up of cerebral endothelial cells (CECs), which cover the inner surface cerebral capillaries and create a selective barrier.[1,5]

Doxepin HCL is Tricyclic antidepressant BCS Class 1 this is used to treat depression, anxiety it is authorized by the United States food and drug administration. It works by increasing the concentration of neurotransmitters norepinephrine (NE) and serotonin(5-HT) in the brain. By delaying their absorption into presynaptic terminal, this process increases the neurotransmission of the neurotransmitters (5HT & NE) and prolongs their availability into the synaptic cleft.[2]

Doxepin HCL is also used in the treatment of migraines as a prophylactic agent.[13,23] Topical creams which contains Doxepin HCL is works as local anaesthetics, aiding in pain management and the treatment of urethral irritation and dysuria.[19,26]

Niosomes are two-layer structure consists of cholesterol and non-ionic surfactant self-assembled to produce nanovesicles. Niosomes are biodegradable, biocompatible and non-immunogenic nanoparticles. Niosomes vesicles are less toxic than the other and niosomes can entrap both hydrophilic and lipophilic solute particles to deliver the drug to suitable target site.[16,22]

Niosomes are primarily composed of two types of ingredients: non-ionic surfactant and additives such as (Cholesterol and charged compounds)[22,24]. The rigidity of bilayer structure is due to the presence of the cholesterol. Niosomes are also referred to as non-ionic surfactant vesicles, are microscopic lamellar structures that form when an alkyl or dialkyl polyglycerol ether class non-ionic surfactant and cholesterol are combined and subsequently hydrated in aqueous solution[18]. Changes in niosomes can be done by addition of cholesterol and they can possess one or more lipid bilayers encapsulating an aqueous core. Niosomes have been created using wide variety of substances, including polyoxyethylene alkyl ether, sucrose ester surfactants[12]. Additionally, they have low toxicity because they are non-ionic, are chemically stable, and don't require any specific conditions or handling precautions[8].

In this current topic of study, the Doxepin HCL niosomal suspension was prepared as in situ nasal gel. With the goal of determining any potential benefits the delivery system may have over the traditional drug delivery technique. Here we combined advantages of in situ nasal gel and niosomal vesicles to create the ideal intra nasal drug delivery formulation.

Oral administration is so simple to take, they are most preferred and practical form of drug delivery. However, when the medication experience substantial hepatic breakdown due to the first pass metabolism, oral drug administration is not preferred.

Therefore, research on alternative medication delivery routes was promoted by the absence of systemic absorption through

the gastrointestinal tract. Such as transdermal, intranasal, subcutaneous, parenteral, and intramuscular. When a medicine is needed to stop an epileptic event quickly, the nasal pathway might be viewed as a backup route to the central nervous system (CNS) because it has some benefits, such as quick absorption and avoiding the hepatic first pass metabolism.

The ability of intra-nasal drug delivery to avoid hepatic metabolism and brain barriers, like the blood brain barrier (BBB), which divides cerebral fluid from systemic blood circulation and is composed tight junctions of endothelial cells of brain, has made it intriguing in the modern era[7,15,20,25].

Using the nasal passage/ route to administer the drug is simple practical method. An advantageous alternate method of administration is the intra nasal drug delivery system, particularly for peptides and proteins. Through oral route less bioavailability of these medicinal substances occurs when they are broken down by the gastrointestinal site's enzymes.[14,28,29,30]

There are three routes by which a drug can be transferred to the brain;

Intracranial delivery, systemic absorption via the blood-brain barrier and intrasinusoidal delivery. Among these three routes intra nasal administration is easy and convenient and it also provides advantages such as quick, non-invasive, and focused drug deliver to the brain through the trigeminal nerve.[9,10,11,27]

Materials :

Doxepin HCL was purchased from Yarrow chem products Ghatkopar west, Mumbai, Maharashtra. Tween 20, Tween 80, Cholesterol, Methanol, Diethyl ether, Carbopol 934, PVP K 30, Polaxomer 407 were supplied by New Neeta Chemicals, Pimpri-Chinchwad, Pune, Maharashtra. Phosphate buffer saline (pH 6.4) was prepared as described by Indian Pharmacopoeia. And were used without any modification.

Formulation of Niosomes :

By employing non-ionic surfactants such as Tween 20, Tween 80 and cholesterol in various ratios, ether injection approach[6] was used to create niosomes containing Doxepin HCl.

Accurately weigh quantity of Cholesterol and Surfactant (Tween 20 / Tween 80) then dissolve it in 6 ml of diethyl ether and 2 ml of methanol which contain weighed quantity of Doxepin HCl.

Then take a syringe, fill the above solution in the syringe and add this in 15ml of aqueous solution at a rate of 1ml/min. maintain the temp. of aqueous solution at 60^0C.[4]

Stir this solution on a magnetic stirrer and maintain temp at 60 – 65^0 C.

Then the formulation sonicated for 15 min in sonicator. Various niosomes batches were formulated in order to choose the best possible result.

Factorial design 2^3 is used to design the formulation F1 to F13.

Table-1. Independent factors and their levels

Independent factors	Low level (-1)	High level (+1)
Concentration of cholesterol	17 mg	25 mg
Concentration of surfactant	17 mg	25 mg
Type of surfactant (Tween 20 / 80)	Tween 20	Tween 80

Table-2. Composition of Doxepin HCl loaded Niosomes.

Batch code	Conc. of cholesterol (mg)	Conc. of surfactant (mg)	Type of surfactant
F1	22	17	Tween 20
F2	25	22	Tween 20
F3	25	17	Tween 80
F4	22	17	Tween 80
F5	17	22	Tween 20
F6	22	25	Tween20
F7	25	17	Tween 80
F8	17	17	Tween 20
F9	17	17	Tween 80
F10	25	25	Tween 80
F11	22	22	Tween 80
F12	22	22	Tween 20
F13	17	25	Tween 80

Characterization of Doxepin HCl loaded niosomes :

Evaluation of vesicle size :

Using optical microscopy, the size and morphology of the vesicles in the sonicated formulations were examined by particle size analyser. (labomed Microscope)

Zeta potential analysis :

Using a zeta potential analyser, the charges existing on the Doxepin HCl loaded niosomal suspension was obtained.

Fourier-Transform infrared (FT-IR) spectroscopy :

The compatibility of Doxepin HCl with cholesterol and tween 20 was evaluated using FT-IR spectroscopy. For each test, 2mg of either pure Doxepin HCl, pure cholesterol, and tween 20, or their physical mixture, was used for characterization.

Entrapment Efficiency (%EE) :

The %Entrapment Efficiency of Doxepin HCl loaded niosomal suspension was carried out using centrifuge technique. Doxepin HCl niosomal suspension were centrifuged at 10000 rpm for 15 min at 4^{o}C using refrigerated centrifuge, in order to disentangle niosomes from non-entrapped drug.

For centrifugation 1 ml of freshly prepared niosomal solution was kept and add it in centrifuge tube and kept it in cooling centrifuge at 10000 rpm for 15 min at 4^{o} C.

After that supernatant layer is taken and additionally diluted using the buffer solution (pH 6.4), by using UV spectrophotometer (Shimadzu, Japan) measure the amount of free drug present in the supernatant layer.

%EE = (A/B) *100

Whereas A = entrapped drug

B = total added drug.

Formulation of Doxepin HCl *loaded niosomal in situ gel :*

For the preparation of *in situ* nasal gel Carbopol 934 (0.3%w/v) and PVP K30 (0.3%w/v) is used here PVP K30 used as a mucoadhesive agent. Mix the above solution in Niosomal solution (10 ml). Then keep this mixture on the magnetic stirrer and after that add slowly Polaxomer 407(18%w/v) while adding polaxomer 407 continuous stirring is required.

After the addition of Polaxomer 407 keep the above dispersion on magnetic stirrer for 10 min and after that add 2-3 drops of triethanolamine into it.

After that store the above dispersion in the refrigerator for 1 day.

Characterization of in-situ nasal gel :

Visual appearance/ colour :

Visual appearance/ colour of the Doxepin HCl loaded niosomal in-situ gel is visually examined and it was found to be white in colour.

pH :

pH of the Doxepin loaded niosomal in-situ gel was observed on digital pH meter.

Gelling temperature :

By heating the gel formulation to a (temp. 1^{o} or 2^{o}C) in the test tube and gently swirling until the gel form, the gelation temperature was determined. When there was no longer any flow observed upon tipping the test tube, gelation was declared to have occurred.

Gel melting temperature :

The gel melting temperature was found by measuring the temperature at which gel starts to flow when the test tube is tilted 90^{0} degrees.

Viscosity / Rheological study :

Viscosity of Doxepin HCl loaded niosomal in situ gel was observed on the digital viscometer (LMDV 60) by using spindle no.4 at 60 rpm.

In-vitro drug permeation study of gel :

A modified Franz diffusion cell was used to evaluate the *in-vitro* drug permeation of the generated batches of niosomal gel formulations across a cellophane membrane. Between glass diffusion cells donor and receptor compartments, a cellophane membrane was placed. The donor compartment was adjusted as designed to ensure that the permeation medium is in contact with the cellophane membrane. A prepared sample equals to the 5mg of Doxepin HCl loaded niosomal in situ gel was stored in the compartment which had a cellophane membrane covering it.

The receptor compartment was loaded with 28 ml of phosphate buffer saline (pH 6.4) and magnetic stirrer was used to keep the temperature of medium constant. To keep the skin condition, after that 4ml of solution from receptor compartment is withdrawn after specific time interval and replace it with

equivalent volume of fresh medium.

After that, the sample was analysed for percent drug permeated from the formulation at 290 nm by UV spectrophotometer.

Figure 1. Franz Diffusion Cell apparatus.

Standard calibration curve :

The UV-visible spectrophotometric study was carried out in a pH 6.4 phosphate buffer solution, with a 200-400 nm range used for the evaluation. λ_{max} was measured in phosphate buffer solution 6.4 at 290 nm.

Table-3. Calibration curve

Concentration (ug/ml)	Absorbance
2	0.031
4	0.22
6	0.503
8	0.72
10	0.964

The R^2 value is 0.9973 which is very close to the 1, it shows that greater accuracy of the curve in describing detectors response.

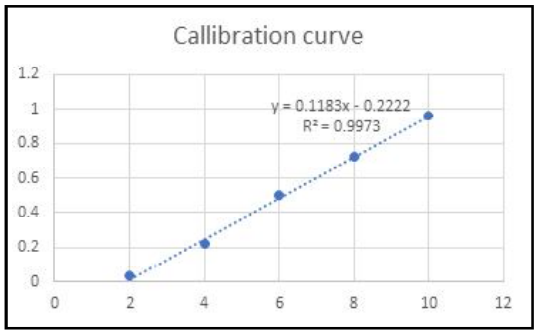

Figure 2. Calibration curve.

Vesicle size and % Entrapment Efficiency (%EE) :

Among the various methods enlisted for the formulation of niosomes, Doxepin HCl niosomes formulated by the ether injection method. The impact of various surfactants and drugs surfactant the effect of cholesterol ratio on Doxepin HCl entrapment in niosomes was investigated.

The vesicles formed by the tween 20 is bigger as comparison to tween 80.

Entrapment efficiency is proportional to the HLB as HLB is low entrapment is more.

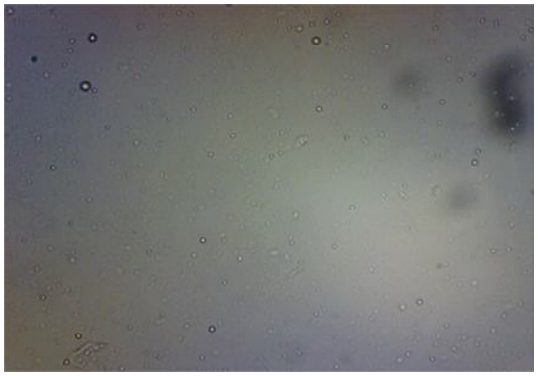

Figure 3. Niosomes microscopic image.

Table-4. Vesicle size and %EE

Batch Code	Vesicle size (nm)	%EE
F1	600.47±150.47	80.9
F2	601.18±111.99	73.59
F3	443.27±63.69	75.2
F4	594.43±152.334	79.82
F5	579.95±146.6	78.95
F6	700.05±200.301	85.31
F7	443.27±63.99	80.1
F8	567.43±156.74	84.68
F9	580.77±142.76	79.86
F10	714.69±183.66	83
F11	422.75±110.75	88.9
F12	441.67±98.37	90.67
F13	673±169.8	83.6

Zeta potential analysis :

The charge on the niosomal vesicle's surface had a negative value, according to the zeta potential study.

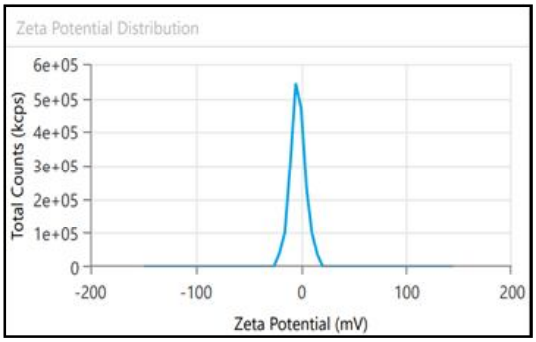

Figure 4. Zeta potential

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-2.861	-	-	-2.861	-2.861
Zeta Peak 1 Mean (mV)	-2.861	-	-	-2.861	-2.861
Conductivity (mS/cm)	4.907	-	-	4.907	4.907
Wall Zeta Potential (mV)	0	-	-	0	0
Zeta Deviation (mV)	7.281	-	-	7.281	7.281
Derived Mean Count Rate (kcps)	6.04E+04	-	-	6.04E+04	6.04E+04
Reference Beam Count Rate (kcps)	363.8	-	-	363.8	363.8
Quality Factor	0.7493	-	-	0.7493	0.7493

Figure 5. Statistical table.

Fourier-Transform infrared (FT-IR) spectroscopy :

The interaction between the drug, other excipients and physical mixture of the final formulation were analysed by using FT-IR spectroscopy. The spectra were observed in the region from 4500 to 500 cm^{-1}.

The FT-IR spectra of pure drug Doxepin HCl is given in fig. 6 and spectra of drug, excipients and physical mixture is given in the fig. 7. Upon spectral analysis, it was found that medication being utilized and the excipients were compatible, indicating safety of created formulation.

FTIR spectrum of Doxepin HCl (fig.no. 6) indicates the presence of N^+-H at 2655 cm^{-1}, $(-CH_2-)_2$ at 752 cm^{-1}, (-C-O-C-) at 1006 cm^{-1} it confirms the presence of Doxepin HCL in the complex.

FT-IR spectrum of the formulation shows a peek at 3489 cm^{-1} for the hydrogen bonded -OH stretching, 1640 cm^{-1} shows the carbonyl stretching, 898 cm^{-1} shows the C-N^+ stretching, 977 cm^{-1} shows the C-C-O symmetric and 1002 cm^{-1} shows the C-C-O asymmetric vibrations of choline chloride, 675 cm^{-1} shows the =C-H out of plane bending 798 cm^{-1} shows C-H out of plane bending.

Differential scanning calorimetry (DSC):

Differential Scanning Calorimetry analysis was carried out on pure form of the drug, and formulation prepared with the excipients. Doxepin HCL shows a sharp pick at 190ºC (fig. 8). while the excipients show peaks at 103ºC. (fig. 9)

It shows that there is entrapment of the drug within vesicles.

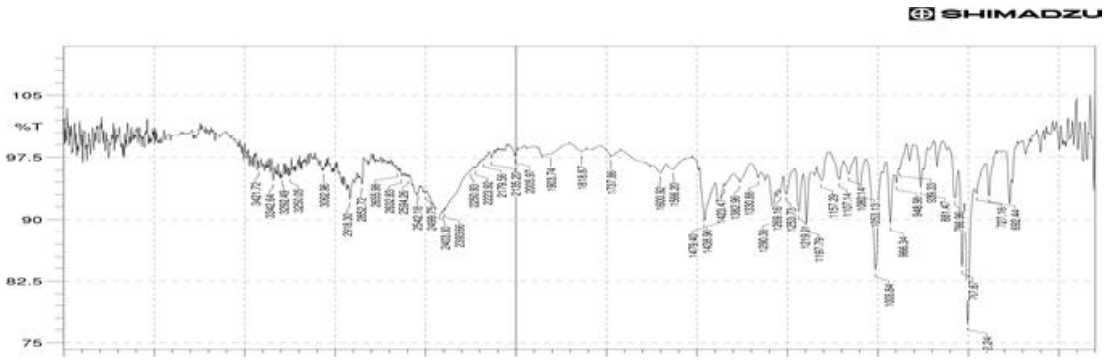

Figure 6. IR spectroscopy of Doxepin HCl.

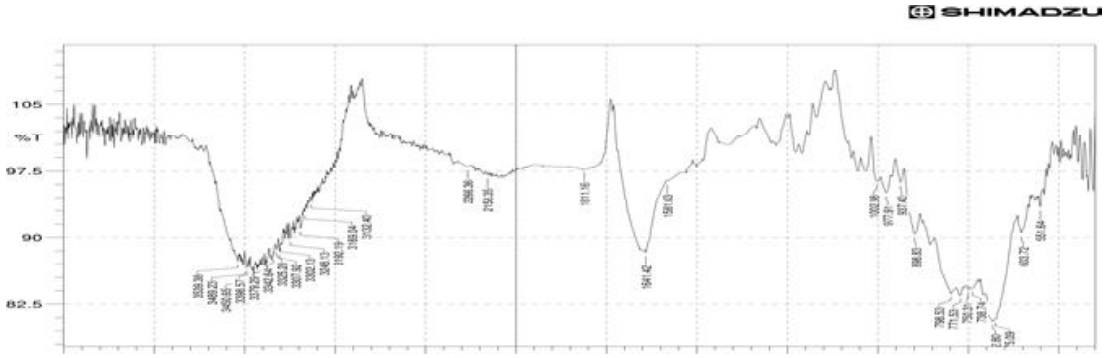

Figure 7. IR spectroscopy of formulation.

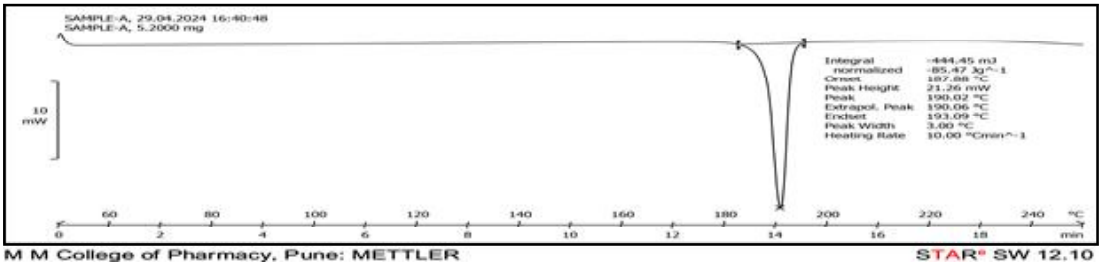

Figure 8. DSC of Doxepin HCl.

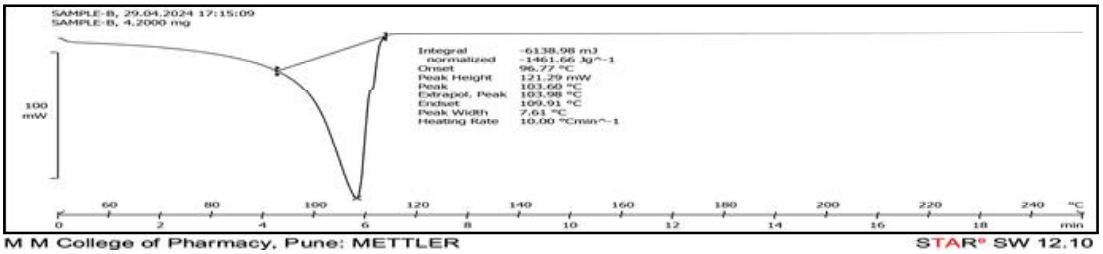

Figure 9. DSC of Formulation.

Colour / visual appearance of gel :

Colour of the Doxepin HCl filled niosomal in situ gel is white.

Figure 10. Doxepin HCl filled niosomal in situ gel.

pH:

pH of the Doxepin HCl loaded niosomal in-situ nasal gel was found to be 6.6±0.2 which is optimum.

Figure 11. pH of the formulation.

Viscosity :

Viscosity of the formulation after removing from refrigerator is shown in fig. no. 12 which is 4941.5 mPas at 60 rpm.

Viscosity of the formulation at body temperature shown in fig. 13 which is more than the refrigerator temperature viscosity. The viscosity shown is 6938 mPas at 60 rpm.

Figure 12

Figure 13

In-vitro drug permeation study :

Table-5. *In vitro* drug permeation

Batch code	Drug release (in %)
F1	65.23
F2	71.29
F3	68.72
F4	64.23
F5	73.65
F6	72.73
F7	68.72
F8	70.15
F9	63.89
F10	69.13
F11	80.12
F12	78.9
F13	73.658

Optimised batch :

After the careful evaluation of the batches for their all the evaluation parameters like vesicle size, entrapment efficiency, viscosity, pH, *in-vitro* drug release and other evaluation parameter.

It was observed that F11 shows the good results.

RSM Effect on Excipients :
Independent variables impact on vesicle size:

The type of surfactant has a major impact on the size of niosomes vesicle. The vesicle size of the two surfactants is different with the same cholesterol content. Niosomes composed of lower HLB value shows small vesicle size niosomes, and surfactant with Higher HLB value shows larger vesicle size. Cholesterol is the component which can influence the physicochemical property and the stability.[4] It was discovered that cholesterol significantly affects the niosomes' particle size, regardless of the type of surfactant used. Yet, the kind of non-ionic surfactant used determined how much the cholesterol content affected the size of niosomes.

Impact of independent variables on the effectiveness of Entrapment Efficiency :

Because cholesterol interacts with non-ionic surfactants, the right amount of cholesterol is added to the niosomes to obtain the most stable formulation.

Non-ionic surfactants are the primary components of the niosomes. Non-ionic surfactant is amphipathic including polysorbates. Due to the way the drugs and surfactant interacts, the niosomal carrier (Tween20/ Tween80/Cholesterol) can greatly boost the drugs entrapment efficiency.

Table-6. Optimised batch.

Batch code	Viscosity	Vesicle size	Entrapment efficiency	Percent drug release
F11	4941.5 mPas	422.75±110.65 nm	88.9 %	80.12 %

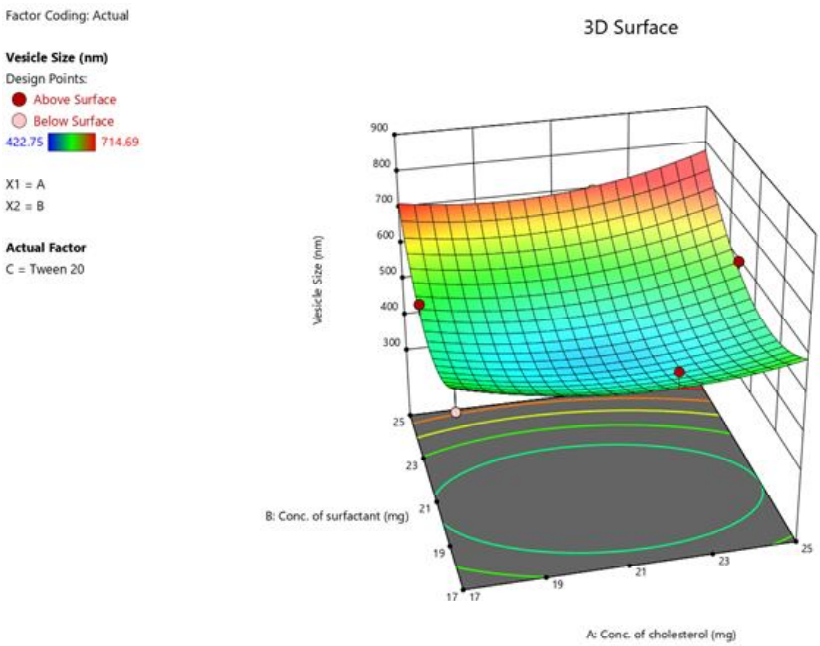

Figure no. 14: - response surface plot showing effect of varying independent variables on particle size.

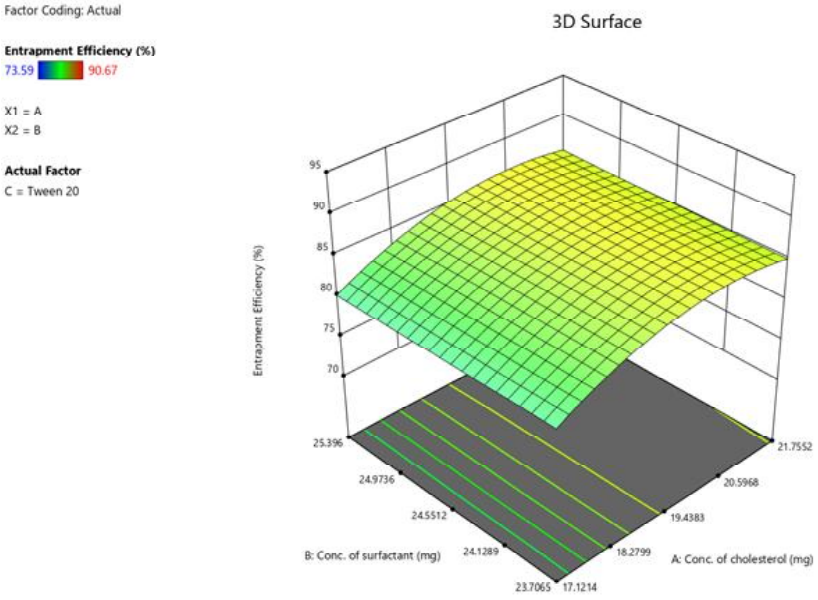

Figure 15. Response surface plot showing effect of varying independent variables on Entrapment Efficiency.

The goal of this study was to create and assess a niosomal in situ nasal formulation of Doxepin HCl by using various surfactant types and different concentration of surfactant and different concentration of cholesterol.

Thus, the use of niosomes showed that nasal to brain administration of Doxepin HCl was a viable alternative to traditional formulations. Doxepin HCl intranasal delivery of drugs has at last been developed successfully. The percentage entrapment efficiency was found to be high when the same ratio of cholesterol and surfactant was measured.

Thus, we can draw a conclusion that a viable method for antidepressant medication Doxepin HCl is the niosomal in situ nasal gel.

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