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## Development of Novel formulation for Intranasal Delivery Containing Desvenlafaxine Succinate

Raghavendra Kumar Divedi\*, Swatantra K. S. Kushwaha, A. K. Rai, Neelottama Kushwaha, Shobhana Srivastava, Divya Dwivedi

Department of Pharmacy, Pranveer Singh Institute of Technology, Kanpur, India

\*Corresponding author: Raghavendra Kumar Divedi, Department of Pharmacy, Pranveer Singh Institute of Technology, Kanpur, India, Tel: +919336430694; E-mail: [raghavendradivedi13@gmail.com](mailto:raghavendradivedi13@gmail.com).

### ABSTRACT

This study was aimed to optimize and formulate desvenlafaxine succinate loaded niosomal *in situ* nasal gel for the treatment of depression. Desvenlafaxine succinate loaded niosomal *in situ* gel was fabricated by Thin Film Hydration method also known as hand shaking method. The present study involves the fabrication, optimization and characterization of the niosomal *in situ* gel. The formulations were fabricated to enhance the bioavailability of lipophilic drug, so the drug can cross the barriers present in the brain without any complications. The barriers present in the brain i.e. cerebro spinal fluid barrier, and blood brain barrier causes hindrance to molecules to permeate the brain but permit the lipophilic molecule to pass. The niosomes were optimized by 32 and each formulation was divided by 22 for the preparation of hydrogel 1% w/v and 1.5% w/v chitosan (for example-in formulation F1 22 is applied then it will be divided into two parts F1G1 and F2G2, 1% w/v and 1.5% w/v chitosan respectively). The particle size of the optimized formulation F1G2 was 342.9 nm. The TEM images of the optimized formulation showed the formulated niosomes vesicles. Maximum entrapment efficiency was found 83.7% of the optimized formulation. *In vitro* release was performed for 8 hours and maximum release shows. Drug releases of the prepared formulations were performed for 8 hours and maximum release shows 82.57%. Zeta potential value of the optimized formulation F1G2 was -22.4 Mv, which shows the stability of the system. Stability study is performed by following the ICH guidelines.

**Keywords:** Hydrogels, pH sensitive, Chitosan, Antidepressant, Desvenlafaxine succinate.

### INTRODUCTION

Depression is one of the known and major psychotic disorders, and it is identified by loss of self-assurance, poor focus, disturbed sleep depressive mood, despondency, misery and suicidal attempts following the lack of interest in the social life. Depression affects round about 16% of the population and is the foremost genesis of suicide in over 60% of cases [1]. Even though depression is not a life-threatening disability, suicides have been recorded in the worst-case scenarios [2].

Venlafaxine is a third generation of antidepressant having an active metabolite known as desvenlafaxine. The efficacy of desvenlafaxine

succinate is comparable to venlafaxine, with lesser side effects [3]. In 2008, Food and Drug Administration (FDA) approved desvenlafaxine for the treatment of major depressive disorder [4]. Desvenlafaxine is also used in the treatment of anxiety, menopause vasomotor symptoms and neuropathic pain [5].

Niosomes are non-ionic surfactants vesicles made up of cholesterol, non-ionic surface-active agents and other lipid surface active agents, they are multilamellar or unilamellar vesicles, by their physical properties and structure niosomes are similar to liposomes [6-10]. In the preparation of niosomes vesicles non-ionic surfactants are used while in liposomes formulation phospholipids are used. Due to non-ionic in nature, niosomes vesicles are widely considered as a suitable choice of liposomes [11, 12]. Niosomes vesicles are less toxic and are able to entrap both lipophilic and hydrophilic solute particles, to deliver the drug at the targeted site [11, 13]. Mainly, niosomes are made by two types of ingredients that are additives (cholesterol and charged molecules) and non-ionic surfactants [11, 14]. Cholesterol provides the rigidity to the bilayer structure of the vesicles. The gamut of surfactants is not applicable for the formulation of niosomes. Number 8 in the HLB (Hydrophilic Lipophilic Balance) demonstrate the maximum entrapment efficiency [2]. In this current study, niosomal suspension of desvenlafaxine succinate was prepared as an *in situ* gel at nasal pH, with an object to reach the possible advantages of the delivery system over the conventional drug delivery system. Here we employed dual properties of both niosomal vesicles and *in situ* gels to formulate an appropriate formulation of intra-nasal drug delivery.

In present days, intra-nasal drug delivery has been fascinating due to its aptitude to bypass first pass hepatic metabolism and barriers present in the brain such as Blood Brain Barrier (BBB), which separates the cerebrospinal fluid and systemic blood circulation made up of brain endothelial cells with tight junctions [3, 10, 14, 15]. Intra-nasal drug delivery system is an auspicious alternative route of administration, especially for drugs of proteins and peptides. By the oral route, these therapeutic agents move to be degraded by the enzymes present in the gastrointestinal site which results in less bioavailability [9, 12, 16, 17].

Three distinct routes of administration *via* the brain to deliver the drugs are intra-cerebroventricular administration, systemic absorption through BBB and intra-nasal administration. The route intra-nasal has been considered as the route of choice for therapeutic agents' delivery to the brain because of various benefits like rapid, non-invasiveness and targeted drug delivery to the brain *via* trigeminal region and the olfactory epithelium and thus minimize the systemic side effects [6-8,18] (Figure 1).

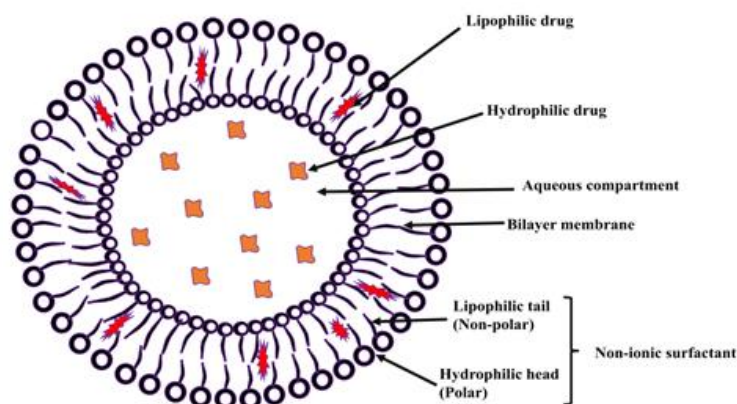


Figure 1: Bilayer structure of Niosome.

## MATERIALS AND METHODS

### Materials

Desvenlafaxine succinate was a gift from Ami Life Sciences, Vadodara (Gujarat) India. Chitosan (Deacetylation degree DDA=80%), dichloromethane, octanol and cholesterol were obtained from Central Drug House (P) Ltd. New Delhi, India and span-60 were obtained from Loba Chemie. Pvt. Ltd. Mumbai, India. Demineralized and double distilled water was used. All chemicals, as well as the reagents used

in this study, were of analytical grade.

### Methods

**Fabrication of niosomes:** Desvenlafaxine succinate (DVS) containing niosomes were fabricated by thin Hand Shaking technique. Statistical technique was selected to formed niosomes. Factorial design 32 is used to design the formulation batches from F1-F9. Accurately weighed quantities of surfactants (span-60), and cholesterol were dissolved in 10 mL of dichloromethane in a round bottom flask indicated in Table 1. Dichloromethane was then evaporated at 40°C by rotary vacuum evaporator to form thin layer on the inner surface of the flask wall. The formed film was left under vacuum at 50°C to remove traces amount of the organic solvent (dichloromethane). The thin film further hydrated with 10 mL, pH 7.4 phosphate buffer solution having 20 mg Desvenlafaxine succinate. The formed suspension was ultrasonicated to obtain multilamellar niosomes [13], for the further study niosomal suspension was kept in refrigerator at a temperature 4°C (Table 1).

Formulation code	Span-60 (mg)	Cholesterol (mg)
F1	20	20
F2	30	20
F3	20	30

**Table 1:** Composition of Desvenlafaxine succinate loaded niosomes.

### Characterization of dvs loaded niosomal suspension

**Fourier-transform infrared (ft-ir) spectroscopy:** Fourier-transform infrared (Spectrum 2 Perkin Elmer) spectroscopy was utilized to determine the compatibility of desvenlafaxine succinate with cholesterol and span -60. 2 mg of either pure desvenlafaxine succinate, pure cholesterol and span -60 or their physical mixture was used for each test for characterization.

**Zeta potential analysis:** Charge present on DVS containing niosomal vesicles were achieved by using Zeta potential analyzer (Litesizer 500). The examined time was set for 1 minute and average zeta potential and charge present on the prepared niosomal vesicles was determined at 25°C.

**Particle size:** The particle size of the DVS niosomal vesicles was performed by particle size analyzer (Litesizer 500).

### Transmission Electron Microscopy (TEM)

The shape of the optimized DVS niosomes was analyzed with the help of transmission electron microscopy; TEM [Hitachi (H-7500)] operated at 120 KV. A dilution of niosomal suspension was placed on a carbon-coated grid for 2 minutes for the adsorption purpose. Then the adsorbed niosomes were negatively stained with uranyl acetate and keep it for air drying for the further visualization of the niosomes [6].

### Entrapment Efficiency

% EE of DVS loaded niosomal suspension was achieved with the help of centrifugation method. For the centrifugation, 5 ml of freshly made DVS niosomal suspension was taken in centrifuge tube and kept in (REMI Instruments Division, Vasai, India) at 9000 rpm for 45 min. The supernatant layer was taken and further diluted with the help of distilled water. The concentration amount of free drug present in the supernatant layer was determined by UV spectrophotometer (shimadzu, Japan). The % EE of DVS loaded niosomal suspension was calculated by using the given equation

Where, A=entrapped drug, and B=total drug added

### **Preparation of dvs niosomal in situ gel**

For the preparation of 1.0% v/v acetic acid solution, 1 ml acetic acid was taken and diluted with distilled water and make-up the volume up to 100 ml. Two hydrogel bases were prepared with the different percentage of chitosan. 1% w/v and 1.5% w/v chitosan were prepared with the help of 20 ml of 1.0% of the acetic acid solution shown in Table 2. Chitosan was dispersed in 1.0% v/v 20 ml of acetic acid solution and stir the solution. The solution was placed on the ultra-sonicator (Hicon, New Delhi, India) for 3 hours. When chitosan was completely dissolved, put it overnight. The hydrogel base and DVS loaded niosomal suspension was taken in (1:1) ratio. In the hydrogel base the prepared niosomal suspension was slowly added with stirring. Put the prepared sample on the ultra-sonicator for 15 minutes and check the pH of the sample.

All the formulation of different batches were evaluated for gelling time and viscosity-to determine the compositions appropriate for the preparation of *in situ* gelling system. The gelation time and conversion of gel into sol were observed. Brookfield Digital Viscometer (Model DV-II + Pro with spindle no-62) in a small volume adapter was taken to determine the viscosity of the all-prepared batches at 20 rpm at room temperature (Table 2).

Batch code	Hydrogel base code	
	1% w/v chitosan	1.5% w/v chitosan
F1	F1G1	F1G2
F2	F2G1	F2G2
F3	F3G1	F3G2

**Table 2:** Hydrogel base code.

### **Characterization of dvs niosomal in situ nasal gel formulation**

Gelling time, viscosity and conversion of gel to sol is given in Table 2.

### **In vitro drug permeation**

*In vitro* drug permeation study of the prepared niosomal gel formulations batches (F1G1, F1G2 to F3G1, F3G2) was studied using a modified Franz glass diffusion cell across cellophane membrane. The cellophane membrane was mounded between the receptor compartment and the donor compartment of the glass diffusion cell. The adjustment of the donor compartment was as fitted so that the cellophane membrane touches the permeation medium. Prepared sample equivalent to 5 mg of DVS was kept in the donor compartment that was attached with the surface of cellophane membrane. 20 ml of phosphate buffer solution pH (7.4) was filled in the receptor compartment and maintained temperature at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  by magnetic stirrer. At appropriate intervals, 4 ml aliquots of the receptor compartment were withdrawn and replaced with the equal volume of fresh medium to maintain a skin condition. The withdrawn sample is filtered through 0.45  $\mu\text{m}$  filter and made an appropriate dilution if needed. Then the sample was analyzed for % drug permeated from the formulation at 222 nm by UV spectroscopy.

### **Stability studies of niosomal hydrogels**

Stability parameters for the formulations are performed to measure of its ability to retain, within the standard criteria and over the period of its storage and use (i.e., shelf life) and all the characteristics of the stability sample should possess at the time of its formulation time. The

specified amount of the niosomal hydrogel is equivalent to 100 mg was placed in a Petri-dish in B.O.D (M/s Khera Instrument Pvt. Ltd. Azadpur Delhi-33) for a period of 1 month. For the testing of drug content, the stability samples were withdrawn at the interval of 7, 14 and 28 days. All the formulations were studied following by the ICH guideline Q1 A (general case) (Table 3).

Study	Temperature and % relative humidity
Long-term	(25 ± 2) °C and (60 ± 5) % relative humidity
Intermediate	(30 ± 2) °C and (65 ± 5) % relative humidity
Accelerated	(40 ± 2) °C and (75 ± 5) % relative humidity
Refrigerator	5°C ± 3°C

**Table 3:** Temperature and % relative humidity.

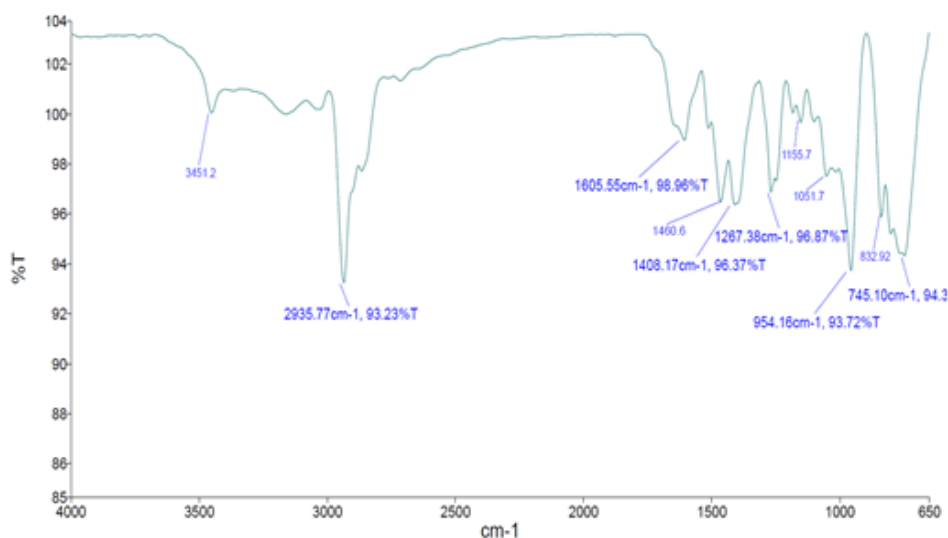
## RESULTS AND DISCUSSION

### Standard calibration curve

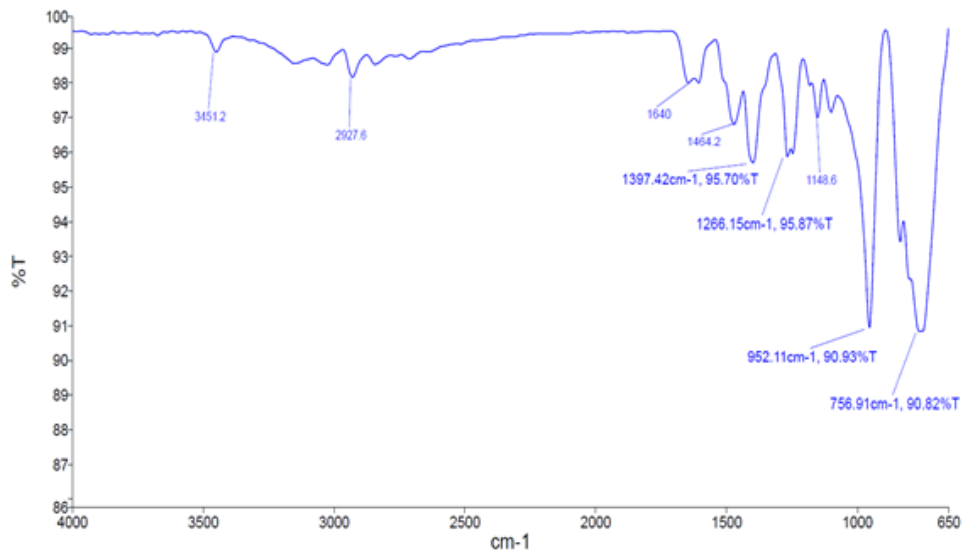
UV-Vis Spectrophotometric study was conducted in phosphate buffer solution pH 7.4 and a gamut is set between 200 nm-400 nm for the examination.  $\lambda_{max}$  was observed in 222 nm in phosphate buffer solution 7.4.

### Fourier-Transform Infrared (FT-IR) Spectroscopy

FT-IR study was performed by using (Spectrum 2 Perkin Elmer), for the understanding of the interaction in the physical mixture of drug and excipients, which are used in the preparation of the niosomal gel. The spectra were observed in the region from 4000 to 650  $cm^{-1}$ . The FT-IR spectra of pure drug (DVS), excipients, and physical mixture of the drug are given in Figure 2, which indicates the spectral peak of pure drug, i.e., DVS; in Figure 3. The spectral interpretation of drug, excipients, and physical mixture of the formulation is given in Table 4. After spectral analysis, it was observed that there is no incompatibility between used drugs with excipients, in result the prepared formulation is safe (Figures 2 and 3, Table 4).



**Figure 2:** FT-IR spectra of desvenlafaxine succinate, cholesterol, span-60 and chitosan.



**Figure 3:** FT-IR spectra of the pure DVS.

S. No.	Functional group	Range (cm <sup>-1</sup> )	Observed frequency (cm <sup>-1</sup> )
1	$\begin{array}{c}     \\ -C-H- \\     \end{array}$	600-1500	1267.38
2	$\begin{array}{c}   \\ -C-H \\   \end{array}$	2850-2960	2935.77
3	$\begin{array}{c}   \\ -N-H \\   \end{array}$	3300-3500	3451.2
4	$\begin{array}{c}   \\ -C-O- \end{array}$	1000-1300	1155.7
5	$\begin{array}{c}   \\ -C=C- \end{array}$	1500-1600	1591.51

**Table 4:** FT-IR frequencies of desvenlafaxine succinate, cholesterol, span-60 and chitosan.

### Zeta potential analysis

The zeta potential analysis revealed that charge present on the surface of the niosomal vesicles having the negative value (Tables 5 and 6, Figure 4).

S. No.	Formulation code	Zeta potential
1	F1	-22.4 ± 0.5 mV
2.	F2	-22.4 ± 0.5 mV
3.	F3	-22.6 ± 0.5 mV

Table 5: Zeta potential analysis.

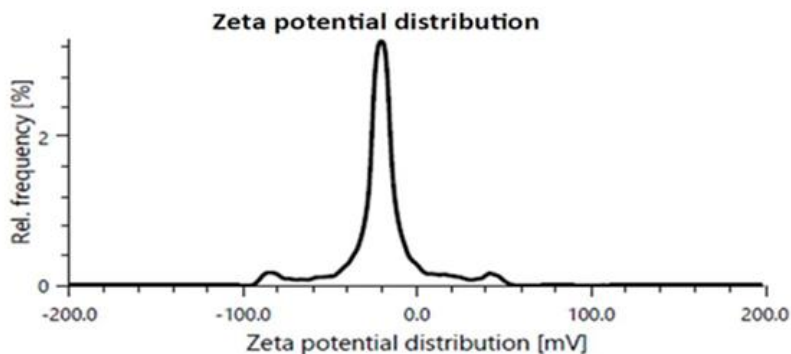


Figure 4: Zeta Potential F1G2. Mean zeta potential -22.4 mV, Mean Intensity-739.2 kcounts/s, Standard deviation -0.5 mV, Filter Optical density -3.2825, Disturbution peak -20.4 mV, Conductivity -0.006 mS/ccm, Electrophoretic mobility -1.7404 cm/vs, Transmittance-77.20%.

**Particle size**

Niosomes formed by Span-60 demonstrated the largest average vesicles in size. Vesicles formed by span-60 are bigger in size in comparison to vesicles formed by Span 80. In addition, vesicles size formed by Tween 20 is bigger in comparison to vesicles formed by Tween 80. This result shows that on increasing the hydrophilicity of the surface-active agents, the vesicles size of the niosomes increase [19] (Figure 5, Tables 6 and 7).

S.No.	Formulation code	Particle size
1	F1	342.9 nm
2	F2	353.6 nm
3	F3	334.1 nm

Table 6: Particle size.

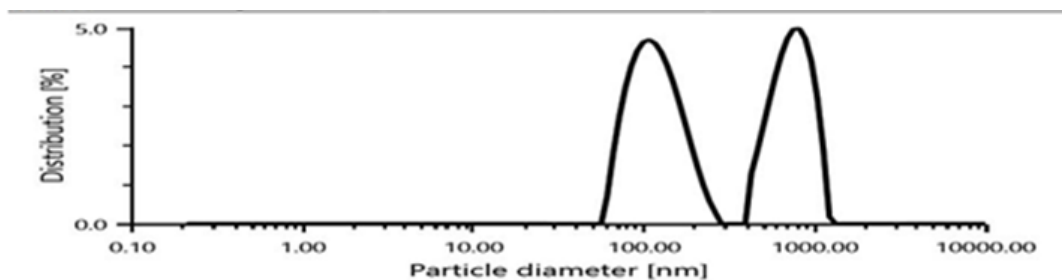


Figure 5: Particle size F1G2.

Hydrodynamic diameter	342.9 nm	Mean intensity	301 kcounts/s				
Polydispersity index	27.90%	Absolute intensity	1291571.5 kcounts/s				
Diffusion Coefficient	1.4 $\mu\text{m}^2/\text{S}$	Intercept g12	0.867				
Transmittance	1.5%	Baseline	1.006				
Particle size distribution peaks	Weighting model	Peak1 (nm)	Peak 2 (nm)	Peak 3 (nm)	Area 1 %	Area 2 %	Area 3%
	Intensity	746.2	121.05	-	45.81	54.19	-
User-defined D-values							
Undersize value	Volume(nm)	Intensity(nm)	Number (nm)				
D10	-	74.49	-				
D50	-	182.3	-				
D90	-	871.8	-				
Undersize value				Undersize span			
Size distribution	D10(nm)	D50(nm)	D90(nm)	(D590-D10)/D50			
Volume	-	-	-	-			
Intensity	79.49	182.3	871.8	4.346			
Number	-	-	-	-			

Table 7: Results.

### Transmission electron microscopy (TEM)

The niosomal suspension was formed with the addition of cholesterol and surface-active agents at distinct molar ratios. The result of transmission electron microscopy showed that niosomal vesicles are well recognized and existing nearly spherical in shape with a smooth surface, having a wide internal aqueous space with uniformity in size [20] (Figures 6-9, Tables 8-11).

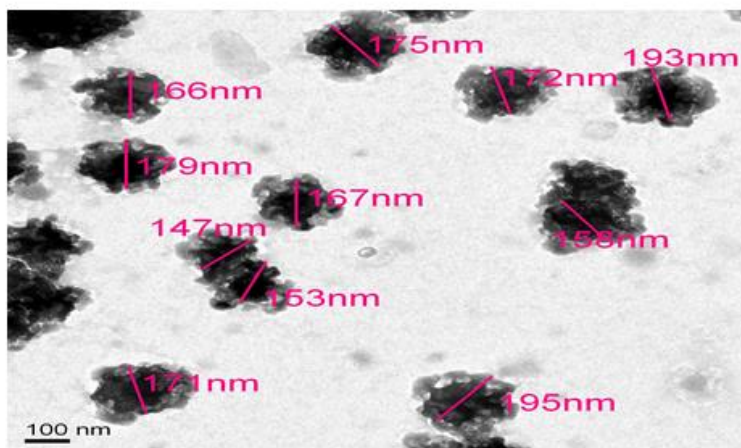
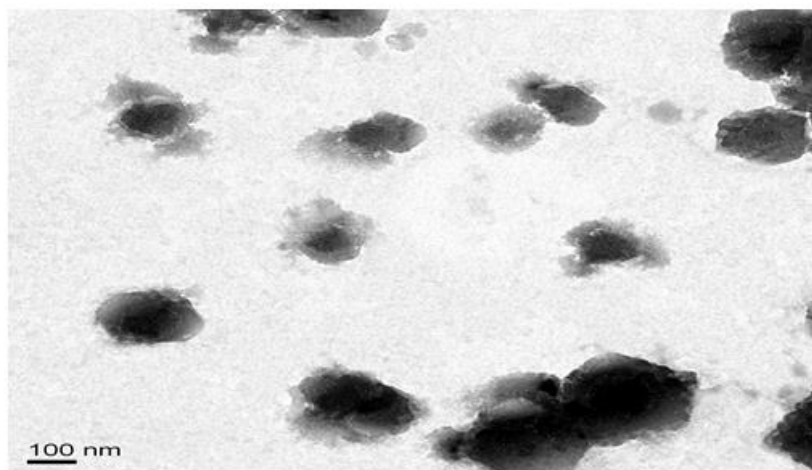
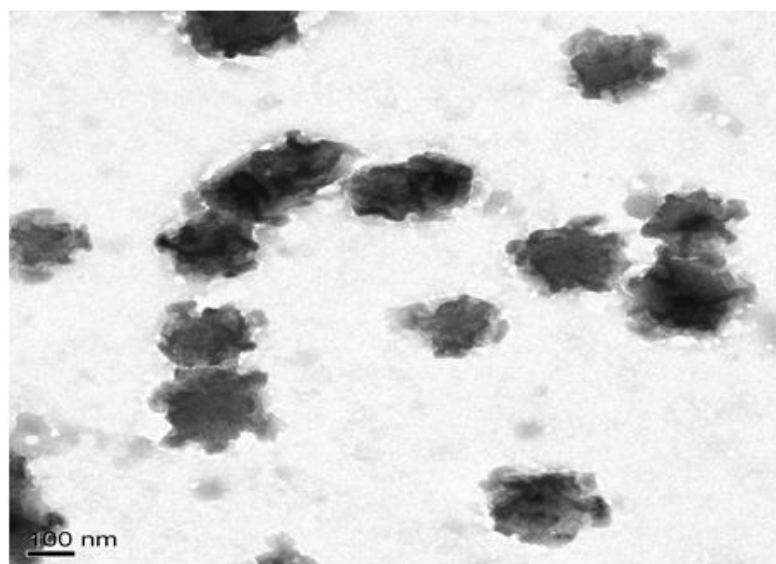


Figure 6: Niosomes formulations F1.





**Figure 7:** Niosomes formulations F1.



**Figure 8:** Niosomes formulations F1.

Batch code	% Entrapment Efficiency	Hydrogel Base code		Gelling time (min)		Viscosity mPa. s at 20 rpm	
		1%w/v chitosan	1.5%w/v chitosan	1%w/v chitosan	1.5%w/v chitosan	1%w/v chitosan	1.5%w/v chitosan
F1	83.7	F1G1	F1G2	5.2 ± 0.32	4.3 ± 0.81	1750	1830
F2	76.2	F2G1	F2G2	9.3 ± 0.32	7.5 ± 0.41	1910	1790
F3	72.5	F3G1	F3G2	8.8 ± 0.75	8.3 ± 0.31	1820	1870

**Table 8:** Entrapment Efficiency Gelling Time, viscosity of Niosomes.

Time in (Hrs)	% Drug release					
	F1G1	F1G2	F2G1	F2G2	F3G1	F3G2
0.5	7.625	10.125	7.625	6.875	6.125	7.375
1	11.8	8.73	9.3	9.725	8.3	8.55
1.5	13.225	13.225	10.725	11.975	10.225	10.225
2	16.15	16.9	13.65	13.65	13.15	12.9
2.5	20.6	22.1	18.1	17.35	18.6	15.35
3	24.95	24.95	24.45	21.95	23.45	19.45
3.5	30.475	30.475	27.975	24.825	25.475	23.725
4	36.35	36.35	33.85	28.85	28.85	28.85
4.5	42.625	42.375	40.125	32.625	32.625	32.375
5	48.3	48.3	45.8	38.3	38.3	34.05
5.5	54.85	57.35	49.85	44.85	44.85	39.85
6	57.575	60.075	55.075	52.575	48.075	42.575
6.5	63.35	65.85	60.85	60.85	53.35	47.35
7	68.95	71.45	66.45	66.45	58.95	55.2
7.5	74.575	74.825	70.075	70.075	67.575	62.575
8	80.075	82.575	77.575	75.075	74.575	71.325

Table 9: *In vitro* release of niosomal hydrogel containing Desvenlafaxine succinate.

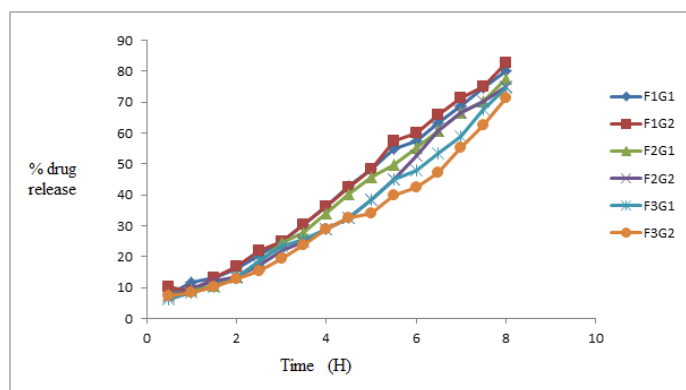


Figure 9: *In vitro* release of niosomal hydrogel containing Desvenlafaxine succinate.

Formulation code	Zero order R2	First order R2	Higuchi model R2	Korsmeyer peppas equation
F1G2	0.999	0.986	0.982	0.997
F2G1	0.998	0.966	0.976	0.993
F3G1	0.992	0.932	0.946	0.984

Table 10: Kinetic data analysis of *in vitro* release data of F1G2, F2G1 and F3G1.

**Stability**

Stability parameters of the formulations were performed in the different temperature and relative humidity variation as per ICH guidelines Q1 A (general) as to achieve the atmosphere for storage condition for the formulations. The observations are given in Table 10. All the formulation passed the stability study as per ICH guidelines [21-25] (Table 10).

S. no	Storage conditions	F1 (%)		F2 (%)		F3 (%)	
		F1G1	F1G2	F2G1	F2G2	F3G1	F3G2
	<b>Long-term</b>						
1	(25 ± 2)°C and (60 ± 5)% relative humidity	96.10%	92.40%	93.70%	94.10%	95.20%	94.80%
	<b>Intermediate</b>						
2	(30 ± 2)°C and (65 ± 5)% relative humidity	93.50%	91.30%	93.20%	93.50%	93.80%	93.20%
	<b>Accelerated</b>						
3	(40 ± 2)°C and (75 ± 5 % relative humidity	90.30%	90.40%	91.80%	92.80%	92.30%	92.50%
	<b>Refrigerator</b>						
4	5°C ± 3°C	98.80%	93.60%	92.20%	95.50%	96.40%	95.10%

Table 11: Stability result.

### CONCLUSION

Thus, the application of niosomes demonstrated the potential for nose to brain delivery of Desvenlafaxine succinate over the conventional formulations. Finally intranasal drug delivery for Desvenlafaxine succinate has been successfully formed. When the same ratio of the surfactant and cholesterol was taken then the %EE was found high, but as well as the amount of the surfactant increases the %EE decreases. All the parameters were maintained properly for the study of stability. All the formulations stability study showed good result that indicate the stability of the formulations.

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