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Formulation and Evaluation of Asenapine Maleate Loaded Niosomes for the Treatment of Schizophrenia

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ABSTRACT

Niosomes are the non-ionic surface-active agent primarily based vesicles. The Key obstacle of Asenapine maleate have oral bioavailability (<2%) and extensive first pass effect. The objective key of the existing work was to formulate. Asenapine maleate loaded niosomes using quality by design and rectify it with some evaluation parameters to increase bioavailability, to lead better therapeutic effect and to minimize the side effects. Formulation of niosomes was done by three methods organic solvent injection method. Factorial design (32) experiments are significantly used to screen and to observe the effect of independent variables cholesterol and span 60 (X1, X2) on dependent variables particle size and entrapment efficiency (Y1, Y2). The results revealed optimized formulation ASP-niosome A2 amongst other formulations which was found lowest particle size 84 ± 5 nm and highest % EE 70 ± 2.0%. *In-vitro* drug release of optimized noisome was found 68 ± 1.20 % at the end of 8 hr and zeta potential was -17.53mV which stabilized the niosomal suspension. Characterization by SEM not only indicated the spherical shape of the niosomes but also confirmed the formation of vesicle. Locomotor activity was found to be significant in *in-vivo* pharmacodynamic study. Pharmacokinetic study carried out and it showed Cmax and t1/2 of 16.12ng/mL and 37.18 hr which is better than reported parameters for drug. Thus, concluded that Asenapine maleate loaded niosomes having effective anti-psychotic activity with increased bioavailability could be prepared successfully by organic solvent injection method using span 60 and cholesterol.

Keywords: *Schizophrenia, Asenapine maleate, Niosome, Quality by Design (QbD).*

1. INTRODUCTION

Schizophrenia is a chronic and severe brain disorder that affects the behavior, thinks and imagination ways of a person. Positive, negative and cognitive symptoms are characterized for this disorder in which, delusion, hallucination, disorganized speech and behavior are more important and considerable.1 Many antipsychotic medications are available in the market but no any single medicament is efficient for schizophrenia patients. The other available treatment options are also not effective in treating. So, these all unsatisfied responses increased the patient noncompliance. Niosomes are non-ionic surface active agents based vesicles. It is formed closed bilayer a structure by the self-assembly of non-ionic amphipiles in aqueous media with the help of physical agitation or by heat energy where medicament is encapsulated in a vesicle. Niosomal structure has hydrophobic parts as well as hydrophilic parts.

Asenapine maleate (ASP) is an atypical antipsychotic multi-receptor neuroleptic drug which shows strong antagonism effects on serotonin, dopamine, noradrenaline and histamine receptors. Sedation in patients is associated with asenapine's antagonist activity at histamine receptors. Their lower incidences of extrapyramidal effects are associated with the up regulation of D1 receptors. This up regulation occurs due to asenapine's dose dependent effects on glutamate transmission in the brain. The sublingual formulation and evaluation of ASP has been studied and already reported that showed an ideal bioavailability of 35%, provided that during its absorption there is no interaction with liquids or foods, mainly fats, over a variable period of time (from 10 min to 4 hr) after it is administered. Asenapine maleate shows a non- linear type of pharmacokinetics in relation to the dose. The double dose of ASP (5-10 mg twice daily) results in 1.7-fold increase in maximum concentration and exposure. ASP may antagonize the targeted disease in the patients. Niosomes of Asenapine maleate could be a very useful

as a drug delivery system for the treatment of brain disorders (Figure 1) or others as well as for the use of other hydrophobic and hydrophilic drugs.

In pharmaceutical industries, factorial design experiments are significantly used to screen and to confirm the product design space. Risk assessment of the manufacturing process is a crucial action in product manufacturing and development, particularly as a backing tool in Quality by Design (QbD). In present research work, niosomes have formulated with different process and formulation variables.

2. MATERIALS AND METHODS

Materials

Asenapine maleate (ASP) was gifted by Lupin Research Park, Pune, India. Cholesterol and Span 60 were purchased from Loba Chemie Pvt. Ltd Mumbai, India. HPLC grade of Chloroform and Acetonitrile and other chemicals were purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

Experimental Animals

To accomplish the objectives of this experiment, Sprague-Dawley rats (200-250 gm) were procured from National Institute of Biosciences, Pune. The protocol and procedure for this study was approved by Institutional Animal Ethics Committee (SCOP/ IAEC/2013-14/179).

Methods

Solubility studies

The solubility of ASP was carried out in the Phosphate buffer saline (PBS pH 7.4) and in other organic solvents. The specific weighed amount of 1 mg of ASP was dissolved in 5 mL of PBS and in other organic solvents and ultra sonicated for 15 min to get a clear solution, then it centrifuged for 10 min at 8000 rpm. After the centrifugation, the supernatant liquid was collected by filtration and further, the concentration of drug was assessed by using UV spectrophotometer (Shimadzu, UV-1800, Japan) at 269 nm for PBS and for other organic solvents at different and specific absorbance values.

Fourier Transform Infrared (FT-IR) spectroscopy studies

The FT-IR spectroscopy was employed to characterize the possible interaction between ASP with cholesterol and ASP with span 60 in the solid state. It was recorded using FT-IR spectrophotometer (Perkin Elmer, Spectrum BX, USA) by using KBr pellet technique in scanning frequency range of 4000-400 cm-1 with a resolution of 40cm-1.

Differential Scanning Calorimetry (DSC) studies

Differential scanning calorimetry (DSC, Stare System, Mettler Toledo) was used to assessed the thermal analysis interactions between ASP with cholesterol and ASP with span 60. Weighed amount of 2 mg of ASP alone and with mixtures were transferred into instrument. Then, it was scanned rate of 10°C /min, over a temperature range of 30- 300°C in the environment of liquid nitrogen (Flow rate 60 mL/min).

Preliminary studies for preparation of niosomes

Hand Shaking (Rotary Evaporator) Method

The specific weighed amount of 5 mg of span 60 and cholesterol were mixed together in round bottom flask. Then mixture was dissolved in chloroform, later on organic phase was evaporated using rotary evaporator instrument under low vacuum. The precipitate thin film was hydrated by using aqueous phase of different weighted amount of ASP such as 5 mg, 7.5 mg and 10 mg (PBS + ASP) on water bath. Later on this niosomal suspension formed was shaken and kept for maturation at 4°C freezer.

Sonication Method

The excess amount of 5 mg, 7 mg and 10 mg of ASP, 5 mg of span 60 and 5 mg of cholesterol were dissolved separately in phosphate buffer saline pH 7.4 and chloroform. The ASP solution was added in the mixture solution of chloroform and then sonicated at 60°C for 3 minutes using a sonicator with a titanium probe.

Organic Solvent Injection Method

Initially 5 mg of span 60 was mixed with 5 mg of cholesterol and later on was dissolved in chloroform. Then mixture was introduced into the solution of phosphate buffer saline (PBS) pH 7.4 containing 5 mg, 7.5 mg and 10 mg of ASP using 21 gauge needle under

constant magnetic stirring at 60°C temperature. Thus the chloroform as an organic solvent was vaporized to form single layered niosomes.

Preparation and evaluation of preliminary trial batches for selection of method of preparation and concentration range of

drug (ASP)

Various trial batches were prepared accordingly for all 3 methods as mentioned in Table 1 and as per the procedure mentioned above. The selection of method was based on PS (nm) and % EE. Cholesterol was used as vesicle stabilizing agent. From the less PS and %EE, span 60 was selected as a surfactant and organic solvent injection was selected as a method that shown as in Table 2.

Preparation of Niosomes

ASP-Niosomes were prepared by organic solvent injection method. The opaque niosome suspension was formedspontaneouslyintheabovementionedconditions. Niosomes were separated by ultracentrifugation (M/S Remi instruments Pvt. Ltd., Mumbai (REMI C-24 BL) at 18,000 rpm for 60 min, at 2-8°C.

Factorial Design of Experiment

In this study, the factorial design 32 was used, where two factors and three levels experimental was performed in all combinations. The amount of Cholesterol (X1) and the Span 60 (X2) were chosen as independent variables, while particle size (PS) and entrapment efficiency (EE) were selected as a response / dependent variables. The factorial design matrix for all batches A1-A9 was shown in Table 3. The resultant data were incorporated into Design Expert V-8 registered software (Stat Ease Inc. Minneapolis USA) and statistically analyzed using polynomial regression equations, ANOVA and 3D response surface methodology.

Characterization of Niosomes

Particle size analysis

The average diameter of ASP-niosomes was determined by Photon Cross-correlation Spectroscopy (PCCS) using Zeta-Sizer Nano-ZS90, Malvern, UK at room temperature. 5 mg of ASP-niosomes was dissolved in distilled water and further it was transferred into sample dispersion unit with continuous stirring to minimize the aggregation.

Entrapment efficiency

To obtain the percentage drug entrapment efficiency of ASP-niosomes, the centrifugation method was applied. In this method, firstly the niosomes was centrifuged at 9000 rpm for 15 min then, the supernatant was analyzed by UV visible spectrophotometer. The percentage drug entrapment efficiency was determined by following equation.

$$\% \text{ EE} = \frac{\text{Mass of drug in niosomes}}{\text{Mass of drug used in preparation}} \times 100 \text{ ---------- } (1)$$

***In vitro* drug release study**

In this method, firstly sample solution of 5 mL of ASP-niosome was kept in a dialysis bag (cellophane membrane, molecular weight cut off 10,000-12,000, Hi-Media, India). Further, it was dipped into the dissolution beaker containing the 900 mL of phosphate buffer (pH 7.4) dissolution medium. The complete apparatus was performed at 50 rpm and 37 ± 0.5°C temperature. As per the required time being, the aliquots were withdrawn from the release medium and replaced with the same up to 8 h. The all sample was assayed in UV spectrophotometrically at 269 nm. Further, the kinetic modelling of Dissolution data was subjected to PCP-Disso-v3 software to find out the release pattern.

Surface morphology

To carry out the surface morphology, Scanning Electron Microscopy (SEM) using JEOL JSM-6360 (Japan) was utilized. In this, a drop of suspension of optimize ASP- niosome was placed on a slide slip before it was fixed, dehydrated and freeze dried then it was scanned at different magnifications.

Validation of optimized preparations/check point analysis

Numerical optimization was performed in order to predict a niosome preparation with particle size less than the 100 nm and maximal % yield from the model generated. By numerical optimization method two check point batches (C1 and C2) were prepared to validate the model.24-26 The coded values of independent variables of two checkpoint batches and their predicated responses are given in result and discussion part, Table 4.

***In-vivo* Studies**

Pharmacodynamics study

1. Spragur-Dawley rats (200-250 gm) were divided into the four groups (*n*= 6)
2. Group I: Normal (control) group
3. Group II: Psychosis induced (Ketamine) group Group III: ASP-API group

4. Group IV: ASP-niosome A2 group

To induce the psychosis activity and behavioral response of all groups of animals, 30 mg/kg ketamine injected *i.p.* daily for five consecutive days. On sixth day, before 30 min of assessment of animal's activity, control group (Group I) was treated with saline and other groups of animals of III and IV were treated *orally* with 0.3mg /kg of asenapine maleate solution (API) and ASP-niosome A2 and the locomotor activity was assessed for 5 min in an open field chamber.

Pharmacokinetic study

For the assessment of pharmacokinetic parameters, group of animals of I, III and IV was selected with administered *p.o.* dose of saline and 0.3mg/kg of asenapine maleate solution (API), ASP-niosome A2 to respective groups. Blood samples were collected by retro orbital route at different time intervals of 1, 2, 3, 4, 5, 6, 7 and 8hr in heparinized tube for a single day. To separate the plasma, the collected blood samples were centrifuged at 8000 rpm for 10 min and stored at -20°C. Then, it was assayed by HPLC analysis under Acetonitrile: potassium dihydrogen phosphate buffer pH 3.2 (60: 40 v/v) and C_{18} column chromatographic conditions.

Statistics and Calculations

Pharmacokinetic parameters were computed with the non-compartmental model strategy. The most extreme plasma drug concentration (Cmax) and the time required (Tmax) by the drug to achieve Cmax were taken from the concentration – time curve. The area under the plasma drug concentration-time curve to the last quantifiable concentration (AUC0-t) was figured using the Trapezoidal rule. The terminal phase was dictated by outwardly inspecting the log-transformed concentration-time curve. The area under the plasma concentration-time curve to infinity (AUC0-∞) was evaluated by joining AUC0-t with AUCt-∞, where AUCt-∞ represents the residual area of the drug from time to infinity and was computed by isolating the last quantifiable plasma drug concentration value by the elimination rate constant (KE). The time required for the drug to diminish its concentration to half (t1/2) was figured as 0.693/KE.

Pharmacodynamics Study

The psychosis activity and behavioral response of all groups of animals were performed in an open field chamber.

3. RESULTS AND DISCUSSION

Solubility studies

The solubility of ASP in pH 7.4 (Phosphate Buffer Saline) and in other organic solvents were determined. In aqueous solution, ASP was showing insoluble, where in chloroform, DCM and methanol, it was found slightly-sparingly soluble. ASP was found to be 2.408 ± 0.7 mg/mL in PBS pH 7.4(n = 3) and the correlation coefficient *R2* value was 0.997 ± 00.13.

FT-IR spectroscopy studies

As depicted in Figure 2 and Figure 3, the FTIR spectra of ASP revealed high intensity broad bands at approximately 650 cm-1(C-Cl stretching), 1093 cm-1 (-O- of Ether), 1485 and 1618 cm-1 (C=C aromatic), 1706 cm-1 (C=O of Acid), 2288 cm-1 (C=N stretching), 2960 and 3038 cm-1 (C-H alkane) and 3417 cm-1 (H-C= aromatic stretching). These peaks have also been observed in a physical mixture of ASP with span 60 and ASP with cholesterol. Thus, the excipients and ASP didn't interact with each other and confirm the formation of a spherical vesicle.

Differential scanning calorimetry studies

Differential Scanning Calorimetry studies (Figure 4) indicated a sharp endothermic peak at 146°C corresponding to melting of pure ASP. The peaks broadening as well as change in relative intensities were observed due to dilution of drug in physical mixtures of ASP with span 60 and ASP with cholesterol. It is concluded the confirmation of complex formation.

Preliminary studies for preparation and evaluation of niosomes

From the less particle size and higher entrapment efficiency, span 60 was selected as a surfactant and organic solvent injection was selected as a method that shown as above in Table 2.

Factorial design and statistical analysis

A 32 full factorial design was constructed to study the effect of the number of independent variables X1, X2 (cholesterol and span 60) on the dependent variables Y1, Y2 (particle size and entrapment efficiency) of ASP- niosomes. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = \beta0+\beta1X1+\beta2X2+\beta12X1X2+\beta11X12+\beta22X22 \quad (2)$$

Characterization of niosomes

Particle size

The average diameter of the prepared ASP-niosomes is summarized in Table 5 and Table 6. The particle size of the prepared

ASP-niosomes was found in the range of 84 ± 5 nm to 296 ± 5 nm. ASP-niosome A2 shows the lowest particle size 84 ± 5 nm and A1 shows highest particle size 296 ± 4 nm that shown in Figure 5.

With increasing in the concentration of cholesterol and span 60 were found to influence or increase the particle size of the prepared ASP-niosomes. The polynomial equation for particle size was found as shown below

Y1 (PS) = +106.56-16.33 X1-12.00X2+71.75X1X2+111.67X12- 22.33X22 (3)

Table 1: Trial Batches of ASP-Niosomes.

Run	Surfactant	Cholesterol: Span 60:Drug	Method	Weight (mg)		
				Cholesterol	Span 60	Drug
T1	Span 60	1:1:1	Organic Solvent Injection	5	5	5
T2	Span 60	1:1:1.5	Organic Solvent Injection	5	5	7.5
T3	Span 60	1:1:2	Organic Solvent Injection	5	5	10
T4	Span 60	1:1:1	Sonication	5	5	5
T5	Span 60	1:1:1.5	Sonication	5	5	7.5
T6	Span 60	1:1:2	Sonication	5	5	10
T7	Span 60	1:1:1	Hand shaking	5	5	5
T8	Span 60	1:1:1.5	Hand shaking	5	5	7.5
T9	Span 60	1:1:2	Hand shaking	5	5	10

Table 2: Evaluation of Preliminary Trial Batches for Selection of Method of Preparation.

Organic solvent injection method			Sonication method			Hand shaking method		
Formulation code	PS (nm)	%EE	Formulation code	PS (nm)	%EE	Formulation code	PS (nm)	%EE
T1 (span60)	506	54	T4 (span60)	758	18	T7 (span60)	7154	33
T2 (span60)	884	67	T5 (span60)	1118	27	T8 (span60)	6908	44
T3 (span60)	1045	84	T6 (span60)	2158	55	T9 (span60)	8700	52

Table 3: Preparation of Asp-Niosomes by 3^2 Factorial Design.

Run	Coded Values		Actual Values (mg/mL)		Asenapine maleate (mg)	Chloroform (mL)	Phosphate buffer pH 7.4 (mL)
	X1	X2	X1	X2			
A1	-1	-1	5	10	5	6	30

A 2	0	-1	10	10	5	6	30
A 3	+1	-1	15	10	5	6	30
A 4	-1	0	5	15	5	6	30
A 5	0	0	10	15	5	6	30
A 6	+1	0	15	15	5	6	30
A 7	-1	+1	5	20	5	6	30
A 8	0	+1	10	20	5	6	30
A 9	+1	+1	15	20	5	6	30

The results of multiple linear regression analysis showed that both the coefficients β1and β2 bear a positive sign. Therefore, increasing the concentration of either cholesterol or span 60 is expected to increase the particle size. The result of analysis of Variance for Y1 (particle size) was shown in Table 6, that indicates the quadratic model was found an adequate model. The three-dimensional response surface plot was shown in Figure 6 and Contour plot Figure 7 indicates the effect of cholesterol and span 60 on the particle size of niosomes.

Entrapment efficiency

The % EE of ASP-niosomes was found in the range of 53 ±4.5 - 89 ± 0.5 %. It was influenced by the amount of cholesterol and span 60 are given in Table 5 and Table 7, depict the results of entrapment efficiency obtained for the prepared ASP-niosomes. Maximum entrapment efficiency observed for ASP-niosome A2 containing 10 mg of cholesterol and 10 mg of Span 60.

By conducting statistical analysis the quadratic model was model was found to be best fit model and polynomial equation is shown below

Y2 (EE) =+65.44-13.00X1-4.00X2 -5.21805E-015X1X 2+4.33X12+1.33X22 (4)

The results of multiple linear regression analysis showed that both the coefficients β1and β2 bear a negative sign. Therefore, increasing the concentration of either Cholesterol or Span 60 is expected to decrease the entrapment efficiency. Three-dimensional response surface plot and Two-dimensional contour plot were shown in Figure 8 and Figure 9, indicate the effect of cholesterol and span 60 on the entrapment efficiency of ASP-niosomes. With increasing in the concentration of cholesterol and span 60 were found to influence or decrease the entrapment efficiency of the prepared ASP-niosomes. Graphical representation of entrapment efficiency and particle size is given in Figure 10.

Table 4: Validation of Model.

Check point batches	Independent Variables		Response variables	Predicted value	Observed value	Residual*	% Error**
	X1	X2					
C1	-0.75	1	Y1 (nm)	93.60	95	1.4	1.5
			Y2 (%)	75.00	74.50	-2.5	-3.33
C2	-0.92	1	Y1 (nm)	115.4	118	2.6	2.23
			Y2 (%)	78.35	77	-1.35	-1.72

*residual = actual (experimental) value− predicted value. **error = [predicted value− experimental value/predicted value] × 100

Table 5: Experimental Design with Corresponding Preparations and Results.

RUN	Coded Values*		Actual Values (mg)		Y1 P.S (nm)	Y2 E.E (%)	PDI	Drug Release at 8 h (%)
	X1	X2	X1	X2				
A1	-1	-1	5	10	296±4	89±0.5	0.25	71±1.20
A2	0	-1	10	10	84±5	70±2.00	0.27	68±0.70
A3	+1	-1	15	10	132±3	62±0.5	0.24	64±1.0
A4	-1	0	5	15	254±5	82±0.5	0.31	73±0.20
A5	0	0	10	15	92±4	65±0.5	0.30	62±0.90
A6	+1	0	15	15	197±3	58±1.00	0.24	63±2.0
A7	-1	+1	5	20	109±4	80±0.5	0.25	72±0.40
A8	0	+1	10	20	99±5	64±0.5	0.31	58±0.70
A9	+1	+1	15	20	232±7	53± 4.5	0.26	55±0.90

(Coded values* -1= low, 0= medium and +1= high concentration)

Table 6: Result of Analysis of Variance for Y1 (Particle Size) For Quadratic Model (Partial Sum of Squares Type - Iii).

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Model	48993.36	5	9798.67	14.26	0.0266	Significant
A-X1	1600.67	1	1600.67	2.33	0.2244	
B-X2	864.00	1	864.00	1.26	0.3438	
AB	20592.25	1	20592.25	29.97	0.0120	
A2	24938.89	1	24938.89	36.29	0.0092	
B2	997.56	1	997.56	1.45	0.3146	
Residual	2061.53	3	687.18			
Cor Total	51054.89	8				

The Model F-value of 14.26 implies the model is significant. There is only a 2.66% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case AB, A are significant model terms.

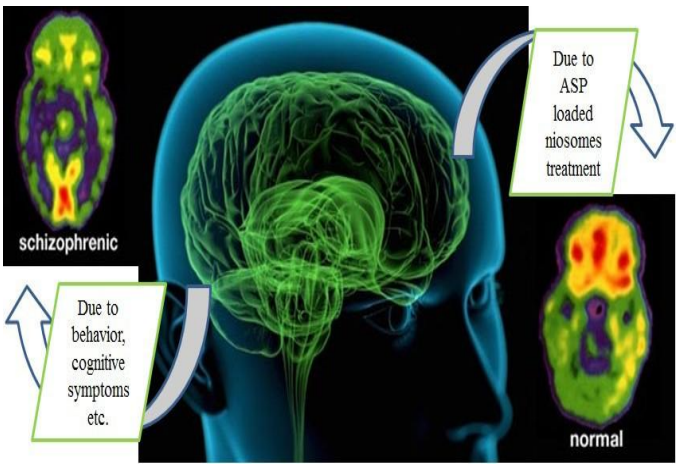

Figure 1: Effect of ASP loaded niosomeson Schizophrenic brain disorder

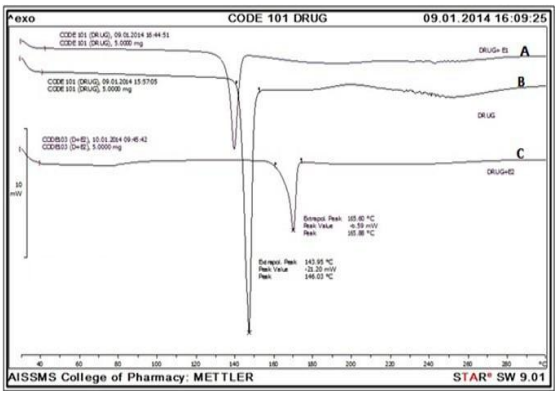

Figure 2: FT-IR spectra of ASP

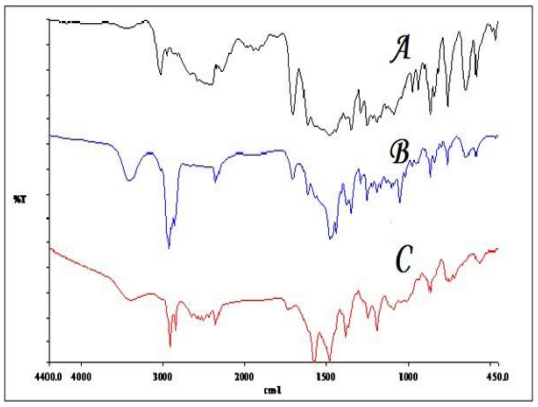

Figure 3: FTIR spectra of ASP (A), ASP + Cholesterol (B), ASP + Span 60 (C).

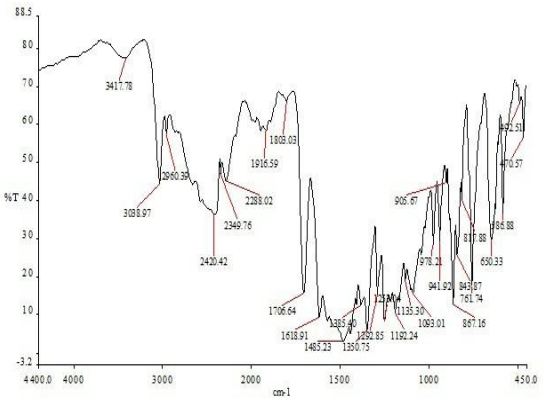

Fig. 4 DSC thermogram of ASP + Cholesterol (A), ASP (B), ASP + Span 60 (C)

***In-vitro* drug release**

The *in vitro* drug release of prepared ASP-niosomes A1– A9 ranges from 55±0.90 to 73±0.20 % and the values are presented in Table 5. The release profile of the ASP from the niosomes in PBS pH 7.4 was shown in Figure 11. All ASP-niosomes showed burst release due to dissolution of surface of ASP from niosomes initially followed by sustained release. The optimized ASP- niosome A2 was showed better cumulative percentage drug release 68 ± 1.20 % at the end of 8 h as compare to the other ASP-niosomes. From the interpretation given in Table 8, the best fitted kinetic model was found to be Korsmeyer-Peppas model (Figure 12).

Surface morphology

The surface morphology of optimized ASP-niosome A2 was spherical shape and crystalline structure as shown in Figure 13.

Zeta potential

The surface charge property and particle stability of optimized ASP-niosome A2 was potential of physical stability as it has zeta potential of -17.53 mV as shown in Figure 14.

Validation of optimized preparations/check point analysis

The experimentally observed values of the responses were compared with that of the predicted values and the prediction error for the two responses were found varying between 3.33 and 2.25 % (Table 4).

***In-vivo* Studies**

Pharmacodynamics study

The antipsychotic effect of optimized ASP-niosome A2 was carried out by pharmacodynamic study. The locomotor activity and behavioral response of rats were assessed by injecting of ketamine (30 mg /kg) i.p. daily for five consecutive days. This dose was found to be increased glutamate outflow in the prefrontal cortex and also sub anesthetic dose of ketamine shows psychtomimetic effects with characteristic behavioral response such as staggered locomotion and increased [14C] 2-DG uptake in the limbic cortical regions, hippocampus, thalamic nuclei and baso lateral nucleus of the amygdale. Psychosis was induced in rats after Ketamine injection. The psychosis was induced and confirmed by the depressant activity, behavioral response like drowsiness and decreased locomotor activity. The psychosis induced rats were then treated

p.o. (0.3 mg/kg) of ASP-niosome A2 and API solution. The locomotor activity was checked for 5 minutes in open field arena chamber at different time intervals. The locomotion's were found significantly improved with time as shown in Table 9 for ASP-niosome A2 and also, behavioral response became as similar as control (Figure 15).

Pharmacokinetic study

The optimized preparation A2 was analyzed in the plasma samples at different time intervals by HPLC (Figure 16) and it was found 4.412 min. The ASP-niosome A2 was detected into the plasma and AUC of ASP- niosome is as shown in Figure 17. The peak plasma concentration of ASP-noisome A2was found increased from 4-11 ng/ mL by *orally* administration, having 35 % bioavailability. The peak plasma concentration obtained of optimized niosome of Asenapine maleate A2 and ASP was 16.12 ng/mL and 4.62 ng/mL, respectively. The half-life t1/2 of the optimized ASP-niosome A2 and ASP was found 37.18 h and 5.21 hr, respectively. This observation was suggested that the formulation of niosomes of ASP is possible which would reduce the frequency of administration of ASP and help to increase patient compliance in the treatment of psychotic disorders like schizophrenia and bipolar disorder I. Pharmacokinetic parameters are shown in Table 10.

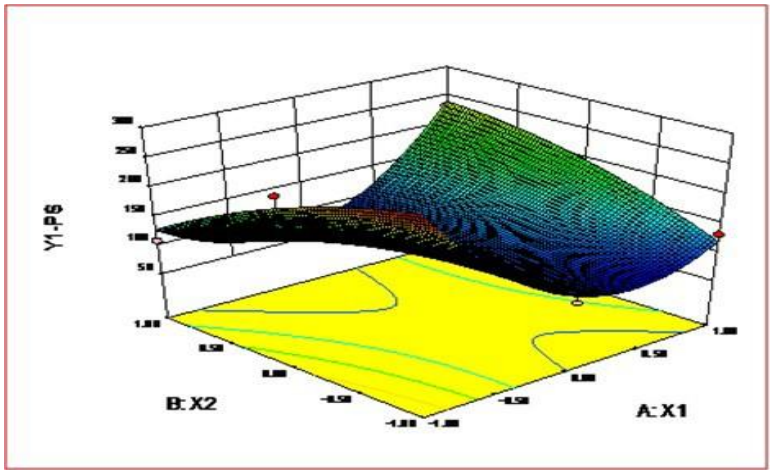

Fig 5: Particle size and Size distribution of ASP-Niosomes

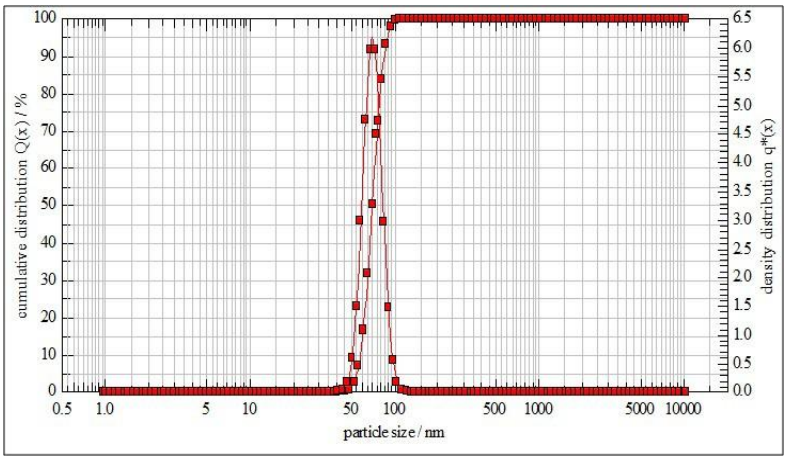

Fig 6: Response surface plot illustrating the influence of Cholesterol and Span 60 on the particle size.

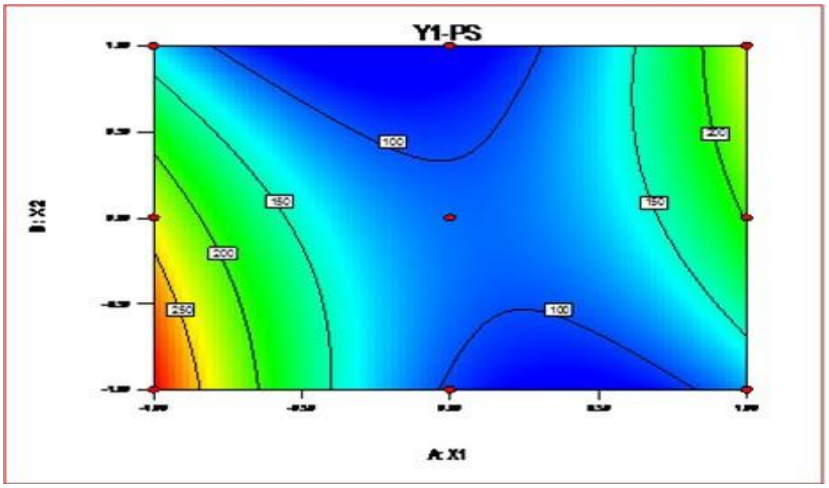

Figure 7: Contour plot illustrating the influence of Cholesterol and Span 60 on the particle size of niosomes.

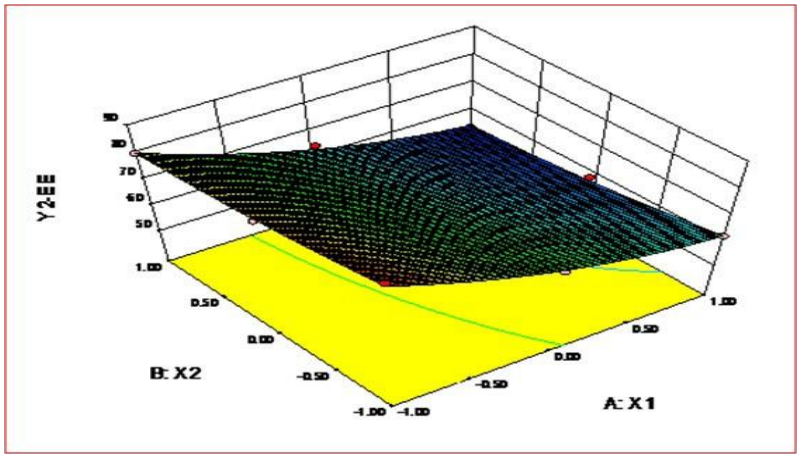

Figure 8: Response surface plot illustrating the influence of Cholesterol and Span 60 on the entrapment efficiency.

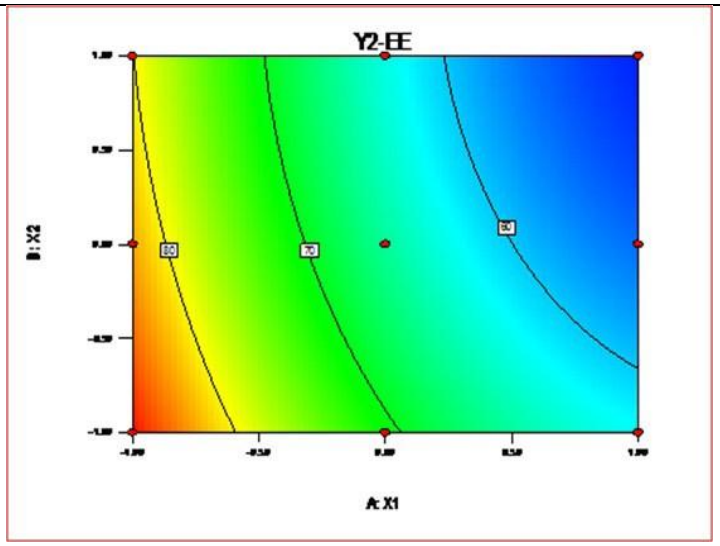

Figure 9: Contour plot illustrating the influence of Cholesterol and Span 60 on the entrapment efficiency.

Table 7: Result of Analysis of Variance for Y2 (Entrapment Efficiency) For Quadratic Model (Partial Sum of Squares - Type Iii).

Source	Sum of Squares	Df	Mean Square	F Value	*p*-value Prob> F	
Model	1151.11	5	230.22	107.17	0.0014	
A-X1	1014.00	1	1014.00	472.03	0.0002	
B-X2	96.00	1	96.00	44.69	0.0068	Significant
AB	2.274E-013	1	2.274E-013	1.058E-013	1.0000	
A2	37.56	1	37.56	17.68	0.0249	
B2	3.56	1	3.56	1.66	0.2885	
Residual	6.44	3	2.15			
Cor Total	1157.56	8				

The Model F-value of 107.17 implies the model is significant. There is only a 0.14% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case 2 A, B, A are significant model terms.

Table 8: Various Model Fittings for Optimized ASP-Niosome A2.

Sr. No.	Model	Correlation Coefficient (R2)	K
1	Zero Order	0.9580	0.4109
2	First Order	0.9598	-0.0042
3	Matrix	0.9860	0.9841
4	Korsmeyer Peppas	0.9942 (*n* =0.6867)	0.7229
5	Hixson Crowell	0.9592	-0.0014

Table 9: Effect of Optimized ASP-Niosome A2 on Locomotor Activity.

Group	Locomotor counts
Control	188.8± 5.94
Disease Control	28 ± 1.31 C
API Application	53± 3.67 #
Formulation	176.5 ± 3.25 @

Table 10: Pharmacokinetic Data of ASP-Niosomes.			
Parameter	**Unit**	**Value of ASP-Niosome A_2**	**Value of ASP (API)**
Cmax	ng/mL	16.12	4.62
Tmax	Hr	2	1
AUC0-t	ng/mL*h	49.38	14.22
t ½	Hr	37.18	5.21

Actual value represents mean ± S.E.M. n=6.

$^{c}P< 0.001$ compared with control rats (ketamine)

$^{\#}P< 0.01$compared with API application rats

$^{@}p< 0.001$ compared with formulation application rats

(one way ANOVA followed by Tukey test)

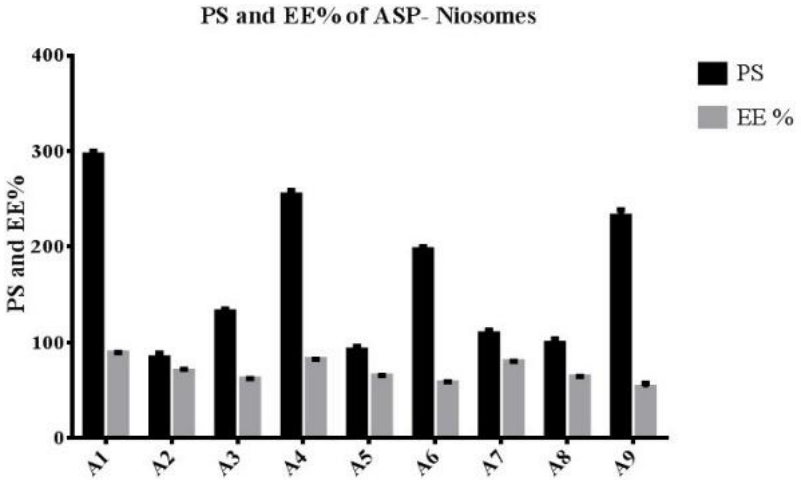

Figure 10: Graphical representation of Entrapment efficiency and particle size of formulations.

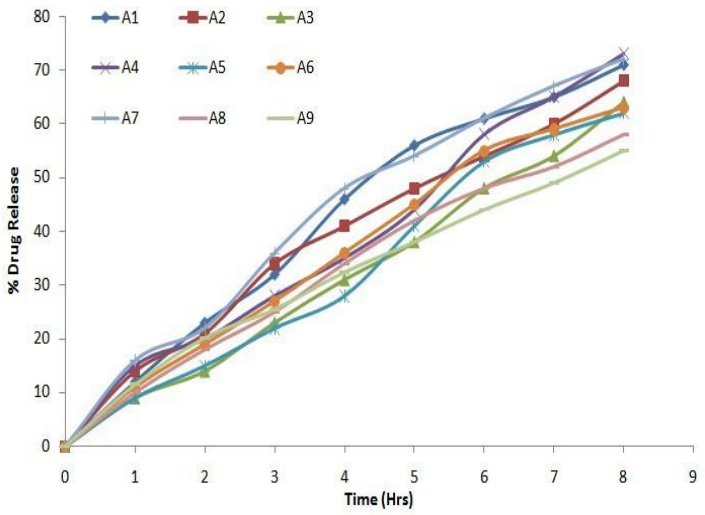

Fig. 11: *In vitro* drug release profile of ASP-Niosome preparation.

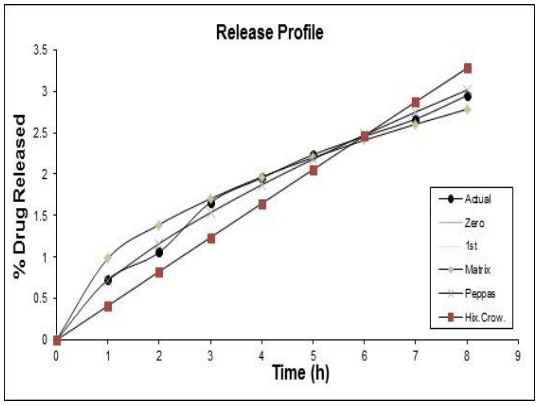

Figure 12: Best fitted dissolution model for optimized optimized ASP -Niosome A2.

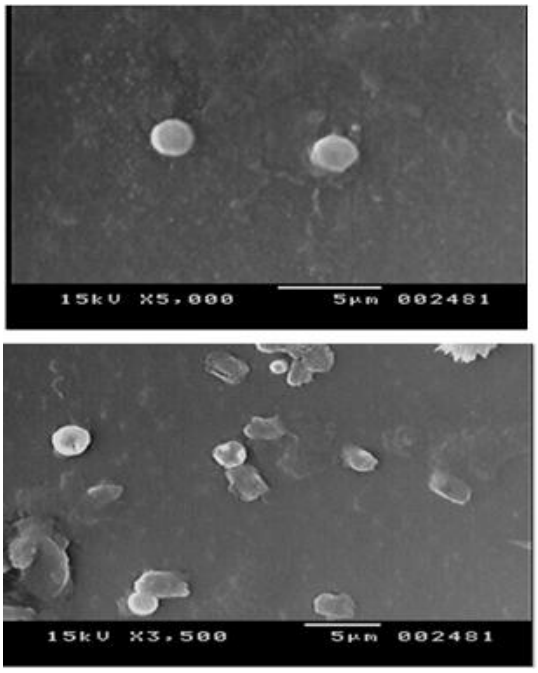

Figure 13: Morphology of optimized ASP -Niosome A2 by SEM Analysis

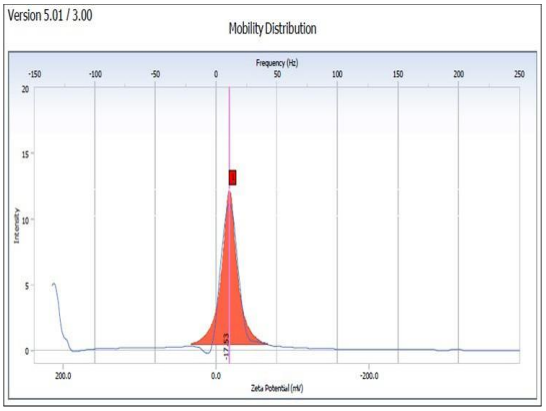

Figure 14: Zeta potential (ζ) determination of optimized optimized ASP-Niosome A2.

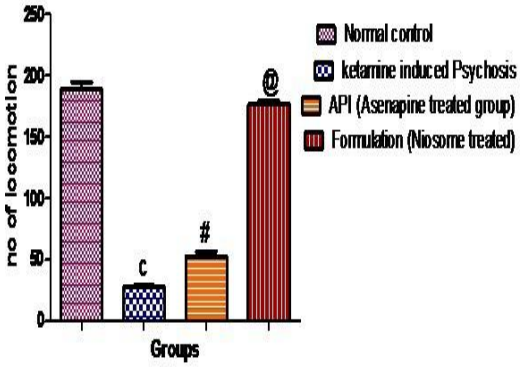

Figure 15: Study of effect of optimized ASP -Niosome A2 on behavioural activity on Rats.

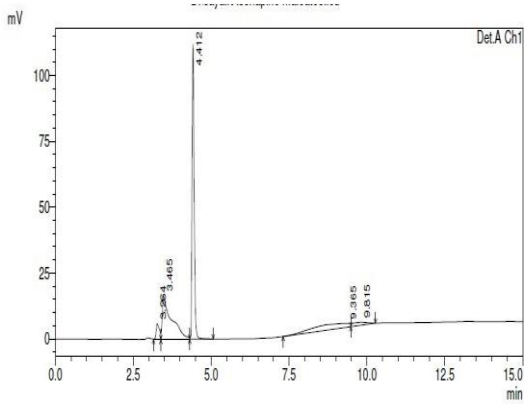

Figure 16: Typical chromatogram of ASP -Niosome A2 (100 µg/mL) using RP HPLC.

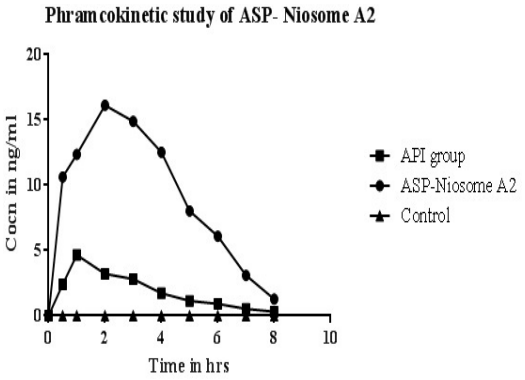

Figure 17: AUC of optimized ASP -Niosome A2.

4. CONCLUSION

Based on the above study we have concluded that selection of a suitable composition of cholesterol and span 60. The desired entrapment efficiency and particle size were achieved by using a 32 factorial design. The optimized ASP-niosome A2 showed better *in vitro* drug releaseas compare to the other ASP-niosomes and also best fitted kinetic model was found Korsmeyer–Peppas (K-P) model and the the drug release is controlled by diffusion process. The surface morphology of ASP- niosome A2 by using scanning electron microscopy reveals that niosomes were spherical in shape and the zeta potential value showed that niosomes have sufficient charge and mobility to inhibit aggregation and are stable in suspension as surface charge prevents aggregation of the particles.The PK-PD studies concluded that ASP- niosome A2 shows greater pharmacokinetic values than reported value of pure ASP. In pharmacodynamic study, locomotor activity and behavioral response was found gradually improved with ASP-niosome A2 showing its improved antipsychotic activity. From the present investigation, it may be concluded that niosomes may increase the oral bioavailability of Asenapine maleate.

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