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Research Article

ASENAPINE MALEATE LOADED SOLID LIPID NANOPARTICLES FOR ORAL DELIVERY

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ABSTRACT

Asenapine maleate (ASM) is a new second-generation antipsychotic approved in August 2009 by U.S FDA for the acute treatment of schizophrenia and manic or mixed episodes associated with bipolar disorder in adults. It shows poor oral bioavailability of < 2% due to extensive first pass metabolism in liver. The present study was aimed at developing and characterizing solid lipid nanoparticles (SLNs) of ASM.SLNs were prepared by solvent injection method by employing Compritol ATO 888 as the lipid matrix and Poloxamer 188 as stabilizer. A 3^2 full factorial design was employed to study the influence of independent variables (amount of lipid and % surfactant) on dependent variables (particle size, and % entrapment efficiency). Optimized ASM-loaded SLNs were further studied for zeta potential, *in vitro* drug release and TEM. Nanoparticles were lyophilized to improve the physical stability and obtain free flowing powder. Mannitol was employed as a cryoprotectant. Lyophilized ASM-loaded SLNs were characterized using DSC and XRD. The optimized ASM-loaded SLNs exhibited mean particle size 318.5 ± 3.2 nm; polydispersity index of 0.255; zeta potential -29.75 ± (-0.92) mV; entrapment efficiency 53.13 ± 1.77 %; drug release extended up to 36 hours. TEM image exhibited spherical smooth surfaced nanoparticles. Accelerated stability studies of optimized ASM-loaded SLNs and Lyophilized ASM-loaded SLNs revealed its stability. The formulation developed shows reduction in dose and dosing frequency and thus reduces dose related side effects and improved patient compliance.

Keywords: Asenapine Maleate, Compritol, full factorial experimental design, Solid lipid nanoparticles, Solvent injection method

INTRODUCTION

The peroral route for administration of drug is consider as the most usual, convenient and safest route which improve patient compliance, decrease complications and lessen cost as compared to other routes of drug administration.[1-3] Despite these productive attributes, therapeutic efficacy of peroral delivery systems is often limited due to physiological constraints along with certain physicochemical properties of drugs. Poor solubility and poor permeability are the main causes for poor oral bioavailability (BA) of the drugs[4].

Physicochemical and metabolic instability in both stomach and liver have a negative influence on the drug concentration in blood. Hepatic first-pass metabolism is another chief cause of poor BA upon peroral administration. However, drug delivery via oral route is still the first choice of formulators keeping in thought the basic biological and pharmaceutical approaches of drug delivery via the oral route[5]. Nanoparticles (NPs) are considered as alternatives to various conventional drug delivery techniques and often used to improve the oral BA of drugs. NPs have been employed to overcome the challenges related with oral drug delivery. A number of nanoparticulate systems based on biocompatible polymers, surfactants, lipids and oils have come to the force, which could be efficiently used to improve the oral BA of drugs either by increasing the drug permeability or by overcoming the first-pass effect and/or P-gp efflux. Apart from these, NPs can also improve the stability of drugs in the gastrointestinal tract (GIT) while modulating the physicochemical and biological properties. In this relation, solid lipid nanoparticles (SLNs) have generated greater interest amongst researchers. SLNs are novel submicron colloidal carriers (50-1000 nm) developed in 1990s. There composition contains lipids like Compritol 888 ATO, Dynasan 112, beeswax, carnauba wax, emulsifying wax, cetyl alcohol, cholesterol butyrate, and cholesterol and stabilized by surfactant. SLNs as colloidal drug carrier combine the advantages of polymeric NPs, fat emulsion & liposomes simultaneously. Like liposomes, they are composed of physiologically accepted biocompatible excipients (fatty acids and lipids). Identical to polymeric NPs, their solid matrix can effectively protect the incorporated drug against degradation under harsh biological milieu and provide the highest flexibilities in the modulation of the drug release profiles. Further, they can be scaled-up easily and produced at large industrial scale[29]. All these constructive attributes make SLNs excellent carriers for oral drug delivery. SLNs can also be administered by other routes such as parenteral, rectal, opthalmic as well as transdermal/topical.

Schizophrenia is a mental disorder often characterized by abnormal social behavior and failure to recognize what is real. It is a complex, chronic, severe, and disabling brain disorder and affects approximately 1% of all adults globally[13-19]. Asenapine maleate is a new second-generation antipsychotic approved in August 2009 by U.S FDA. It is used in the acute treatment of schizophrenia and manic or mixed episodes associated with bipolar disorder in adults. It thought to be due to antagonist activity at dopamine (D2) receptors and serotonin (5-HT2A) receptors the mechanism of action for Asenapine, although unknown. In addition, for other dopamine (D1, D3, and D4), serotonin(5-HT1A, 5-HT1B, 5HT2B, 5-HT2C, 5-HT5, 5-HT6, and 5-HT7), adrenergic (α1 and α2), and histamine (H1) receptors the drug has also high antagonist activity. The oral bioavailability of the drug is less than 2% due to high hepato-gastrointestinal first-pass metabolism[20-24].

The aim of the present investigation was to formulate and characterize SLNs of Asenapine maleate. The SLNs of Asenapine maleate were formulated using Glyceryl behenate (i.e. Compritol 888 ATO) as the solid lipid matrix and Poloxamer 188 as surfactant. Asenapine maleate loaded SLNs were prepared using solvent injection method and optimized for independent variables (amount of lipid and % of surfactant) in order to achieve desired particle size with maximum percent entrapment efficiency (% EE) (dependent variables) using 3^2 full factorial design using Design Expert Software 8.0.7.1.

MATERIALS AND METHODS

Asenapine maleate and Compritol ATO 888 were obtained from Dr. Reddy's Laboratory Ltd., Hyderabad, India and Gattefosse, Mumbai, India respectively. Poloxamer 188 was purchased from S.D Fine Chemicals, Mumbai, India. All other chemicals and reagents were of analytical grade. Design Expert 8.0.7.1 software was used to optimize the formulation.

Preliminary trials

For screening of lipid and surfactant as well as to fix the stirring speed and stirring time preliminary trials were done. Particle size and entrapment efficiency were affected by these factors used as criteria for screening. Compritol ATO 888 and Poloxamer 188 were selected as lipid and surfactant respectively whereas stirring speed and stirring time were fixed at 400 rpm and 30 min. respectively.

Preparation of ASM-loaded SLNs

For the preparation of ASM-loaded SLNs, previously reported solvent injection method was employed[28]. Briefly, accurately weighed Asenapine maleate (30 mg) and Compritol ATO 888 (300-600 mg) were dissolved in 10 ml of isopropyl alcohol, to form the lipid phase of the emulsion. The aqueous phase was prepared by adding specified amount of Poloxamer 188 to deionized water. Lipid phase was rapidly injected (50 drops/min) into the 30 ml of aqueous phase under continuous stirring at 400 rpm for 30 min using a magnetic stirrer. Subsequently, the dispersion was subjected to heating at 50±5 °C under continuous stirring (at 400 rpm) until the organic phase evaporated. Following which, the clear dispersion was immediately cooled in an ice-bath with constant stirring at 400 rpm to obtain ASM-loaded SLNs.

Experimental design

A 3^2 full factorial design was selected to optimize the response of the variables. There are two factors such as amount of lipid (mg) (A) and amount of Poloxamer 188 (%) (B). Their used were varied, and levels-low, medium and high were suitably coded as -1, 0 and +1 respectively. Particle size (Y_1) and % entrapement efficiency include in response variables.

In this design, experimental trials were performed at all 9 possible combinations[25]. A total of 11 batches were prepared, which included two center point batches. All other formulation variables and processing variables were kept invariant throughout the study. The statistical software package and Design-Expert (version 8.0.7.1) was used to build the design matrix. the factors and their respective levels indicates the quantitative formula of the batches. A statistical model incorporating interactive and polynomial terms is used to evaluate the response.

$$Y = b_0 + b_1 A + b_2 B + b_{12}AB + b_{11}A^2 + b_{22}B^2 \quad (1)$$

In equation 1, Y is the dependent variables, namely, particle size (Y_1), and % entrapment efficiency (Y_2). Y indicates the quantitative effect of the independent variables A and B; b_0 is the arithmetic mean response of the nine runs; b_1 is the estimated coefficient for the factor A. The main effects (A and B) represent the average result of changing one factor at a time from its low to high value. The interaction term (AB) shows how the response changes when two factors are simultaneously changed. The polynomial terms (A^2 & B^2) are included to investigate non-linearity. The influence of independent variables analyzed by three dimensional response surface plots and contour plots which are produce by simplified models.

Lyophilization of ASM-loaded SLNs

The SLN dispersion was lyophilized using a Heto Power Dry®LL1500 Freeze Dryer (Thermo Electron Corporation, Denmark). The flowing procedure was used: 2% w/v Mannitol solution was added to the nanoparticle dispersion and then manually filled in 2 ml glass vials. The vials were partially stoppered using lyo rubber stoppers. First, the shelf temperature was reduced from 5 to −70 °C at a rate of 1 °C/ min. The pressure was 60 mT (= 0.08 mbar). These parameters were held for 6 h. For primary drying, the temperature was increased from −70 to −25 °C at 0.5 °C/min, whereas pressure remained unchanged. With termination of the primary drying, the secondary drying was performed by increasing the temperature at a rate of 0.2 °C/min to 25 °C. This temperature was held for 6 h at a pressure of 60 mT (= 0.08 mbar) to obtain free flowing powder. Lyophilized powders were stored in air-tight glass vials stoppered under vacuum and stored at room temperature until further use[26].

Drug content

One ml of ASM-loaded SLNs was taken into 10ml volumetric flask and volume was made up with methanol. It was sonicated for 5 min in bath sonicator and centrifuged at 3000 rpm for 10 minutes. The solution was filtered through0.45 µ membrane filter. The filtrate was suitably diluted and analyzed spectrophotometrically at λmax 270 nm using UV spectrophotometer (UV-1800, Shimadzu, Japan).

Particle size, polydispersity index and Zeta potential

Lyophilized ASM-loaded SLNs were dispersed in deionized water used to measure Particle size and Zeta potential was measured by a Malvern Zetasizer 3000 (Malvern Instruments, UK). The measurement of particle size was based on photon correlation spectroscopy. Polydispersity index was studied to determine the narrowness of the particle size distribution. To determine the surface charge of SLNs zeta potential was used. The zeta potential was determined using electrophoretic light scattering (ELS) at25 °C with electric field strength of 23 V/cm using Zetasizer nano ZS. All the measurements were carried out for three times.

Percentage Entrapment Efficiency (%EE)

The ratio of amount of entrapped drug to the amount of total drug used for preparation of nanoparticles is called as entrapment efficiency. Saturated sodium chloride solution was added to 2 ml of ASM-loaded SLNs to precipitate the nanoparticles[27]. The samples were placed in Eppendorf tubes and subjected to centrifugation (Remi Instrument Ltd., Mumbai, India) at 10,000 rpm for 30 min at 4 °C. After centrifugation, the clear Supernant (I) and solid residue were collected. The solid residue was dispersed in 20 ml of de-ionized water to dissolve the free drug

adsorbed on the surface of ASM-loaded SLNs. The sample was further centrifuged and the supernant (II) was added to the initial supernatant (I).The supernant was suitably diluted and analyzed spectrophotometrically at 270 nm. The entrapment efficiency was calculated by equation,

$$(\%\ EE) = \frac{\text{Wt.of drug used in formulation} - \text{Wt.of unbound drug in supernatent}}{\text{Wt.ofdrug used in formulation}} X \frac{}{100}$$

Contour plots and surface response plots

Contour plots and surface response plots are diagrammatic representation of the values of the response use in explaining the relationshipbetween independent and dependent variables. An experimental response and a set of input variables relation shown by Response surface methodology (RSM). RSM sets a mathematical trend in the experimental design for determining the optimum level of experimental factors required for a given response. The reduced models were used to plot two dimensional contour plots and three dimensional RSM using STATISTICA software at the values of A and B between -1 and +1 at predetermined value of particle size(Y_1) and % entrapment efficiency (Y_2).

In vitro drug release study

Quantitative *in vitro* release of drug from ASM suspension and ASM-loaded SLNs was determined by diffusion technique. A Keshary-Chein glass diffusion cell with a donor phase surface area 1.13 cm^2 and receptor phase volume 20 ml was employed. A dialysis membrane (Himedia, Dialysis Membrane– 150, molecular weight cut off between 12,000 to 14,000 Da) separated the donor and receptor compartments. Accurately weighed samples containing ASM equivalent to 10 mg was placed in the donor compartment whereas 20 ml of phosphate buffer pH 6.8 was used as receptor medium. The entire system was kept at 37° ± 1 °C with continuous magnetic stirring at 75 rpm. Throughout the release studies, aliquots of5 ml were withdrawn at predetermined time intervals (0.5, 1, 2, 4, 8, 12, 20, 24, 32, 36 h), filtered through membrane filter and analyzed spectrophotometrically at λmax 270 nm. To compensate for the loss due to sampling and to maintain the sink condition subsequently withdrawn aliquots were replaced with equal volume of fresh buffer solution. The measurements were carried out in triplicate and cumulative percentage drug release was calculated. The data was statistically analyzed using the software Sigmastat (Sigma Stat, USA).

Drug release kinetics

The drug release data was fitted to various kinetic models like zero order, first order, Higuchi, and Peppas equation, which are widely used in understanding the kinetics and mechanism of drug release. The following graphs were plotted: Qt vs. t (zero order kinetic model); log (Q0-Qt) vs. t (first order kinetic model,) and Qt vs. square root of t (Higuchi model) and log $Qt/Q\infty = n \log t + k$ (Peppas equation), where Qt is the amount of drug released at time t and Q0 is the initial amount of drug present. $Qt/Q\infty$ is the fraction of drug released after time t with respect to amount of drug released at infinite time, k is the release rate constant and n is the diffusional exponent which characterizes the transport mechanism.

Differential Scanning Calorimetry (DSC)

Thermograms of ASM, Compritol ATO 888, physical mixture of ASM and Compritol ATO 888and ASM-loaded SLNs were recorded using a DSC (DSC-60, Shimadzu Instruments,

Japan). 10 mg samples were placed in aluminum pans and heated from 25 °C to 300 °C at a scanning rate of 10 ˚C/min under nitrogen flow rate of 20 ml/min. An empty aluminum pan was used as reference. The instrument was calibrated with an Indium standard.

X-Ray Diffraction Studies (XRD)

XRD studies of ASM, Compritol ATO 888, physical mixture of ASM and Compritol ATO 888 and lyophilized ASM-loaded SLNs were determined using X-Ray Diffractometer (Bruker, Germany). XRD patterns were recorded using Cu K α radiation (40 kV, 40 mA) and scanned over a 2θ range of 10-70°. The scanning was carried out at 1.2 °C/min and at 25 °C. Obtained X-ray diffractograms were analyzed with DIFFRAC plus EVA (version 9.0) diffraction software.

Transmission Electron Microscopy (TEM) of optimized batch

The microstructures of shape of ASM-loaded SLNs were observed under transmission electron microscopy [Hitachi (H-7500) 120 kV]. Diluted dispersion of ASM-loaded SLNs (20 µL) was dropped on a copper grid coated with carbon film and allowed to stay for 10 min until air dried. After complete drying, 2% w/v phosphortungstic acid solution n was dropped onto the grids with several replications for negative staining and dried at room temperature.

Accelerated stability studies

The optimized formulations were studied for their stability and their potential to withstand atmospheric/environmental changes. The lyophilized samples and aqueous dispersion (without freeze drying) were stored at 4 °C; 25 ± 2 °C/60 ± 5% RH and 40 ± 2 °C/75 ± 5% RH. Samples were withdrawn at 1, 2 and 3 months' time interval and analyzed for mean particle size, and drug content. The study was performed for three times.

RESULTS AND DISCUSSION

Formulation development of ASM-loaded SLNs

The formulation development of ASM-loaded SLNs for oral delivery attentive is dealt in the present work. By the preliminary batches, Compritol ATO 888 and Poloxamer 188 were selected as lipid and surfactant respectively. While stirring speed and time were fixed at 400 rpm and 30 min respectively. The levels at which factors will be studied based on the preliminary batch. A simple, economical and reproducible solvent injection technique was accepted for preparation of ASM-loaded SLNs. The prepared nanoparticles were then lyophilized using mannitol as cryoprotectant. The effects of formulation variables viz. amount of lipid (A) and % of Poloxamer 188 (B) were studied using 3^2 factorial design. The particle size and %EE for the 11 batches (B1 to B11) showed a wide variation and were found in the range of 240-400 nm and 25-60% respectively. The response variables depend upon the selected independent variables.

Table 1 Coding of actual values of variable

Translation of coded values in actual units			
Independent variables	**Variable level**		
	Low (-1)	**Medium (0)**	**High(+1)**
Amount of Lipid (mg) (A)	300	450	600
Amount of Poloxamer 188 (%) (B)	0.5	1	1.5
Dependent variables			
Y_1=Particle size			
Y_2=% Entrapment efficiency			

Table 2 Formulation of SLNs batches

Ingredients	**Batches**										
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10***	**B11***
Asenapine Maleate (mg)	30										
Compritol ATO 888 (mg)	300	450	600	300	450	600	300	450	600	450	450
iso-Propyl alcohol (ml)	10										
Poloxamer 188 (%)	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5	1	1
Deionised water (ml)	30										

*B10 and B11 – center point batches

Table 3 Evaluation parameters of batch B1-B11 (Mean ± SD) (n=3)

Batch	**Particle size (nm)**	**PDI**	**% Entrapment efficiency**	**Drug content (%)**	**Zeta Potential**
B1	326.7 ± 4.80	0.429 ±0.045	34.90 ± 2.32	103.68 ± 1.31	-25.45 ± (-3.17)
B2	341.4 ± 5.08	0.423 ±0.060	49.49 ± 1.44	99.16 ± 3.34	-21.26 ± (-2.28)
B3	393.4 ± 4.96	0.266 ±0.008	59.97 ± 2.88	98.92 ± 2.16	-22.18 ± (-1.46)
B4	269.0 ± 6.55	0.481 ±0.021	32.78 ± 1.43	104.14 ± 2.70	-27.61 ± (-2.20)
B5	307.7 ± 7.14	0.330 ±0.008	44.08 ± 4.17	101.30 ± 3.01	-24.24 ± (-1.89)
B6	315.2 ± 3.33	0.441 ±0.012	54.94 ± 3.21	113.60 ± 1.20	-27.90 ± (-3.68)
B7	243.3 ± 2.84	0.426 ±0.007	27.60 ± 3.71	105.58 ± 0.60	-34.72 ± (-2.95)
B8	253.9 ± 5.12	0.482 ±0.036	41.20 ± 2.02	102.17 ± 2.25	-32.75 ± (-4.01)
B9	257.1 ± 6.05	0.445 ±0.026	50.95 ± 4.27	101.38 ± 1.49	-35.87 ± (-4.79)
B10	315.8 ± 7.71	0.361 ±0.015	45.50 ± 3.01	102.49 ± 1.45	-29.24 ± (-1.89)
B11	301.6 ± 4.63	0.349 ±0.018	45.32 ± 3.64	102.97 ± 0.90	-28.65 ± (-1.89)

Table 4: Summary of results of multiple regression analysis for response Y_1 & Y_2

Dependent variable	**Particle size (Y_1)**		**%EE (Y_2)**	
	P value	**Coefficient**	**P value**	**Coefficient**
Intercept	-	+ 303.37	-	+45.15
A	0.0059	+21.12	< 0.0001	+11.76
B	0.0001	-51.20	< 0.0001	-4.10
AB	0.0659	-13.23	0.3242	-0.43
A^2	0.6173	-3.77	0.0246	-1.57
B^2	0.8117	+1.78	0.8678	-0.087

Table 5: Comparative cumulative % Drug Release data of formulation (B1-B11) (Mean ± SD) (n=3)

Time (hour)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**
0	0	0	0	0	0	0	0	0	0	0	0
0.5	34.16 ± 2.77	28.60 ± 0.56	18.78 ± 0.83	38.94 ± 1.28	34.49 ± 3.13	25.36 ± 0.44	41.12 ± 1.83	36.31 ± 1.21	31.72 ± 0.61	35.03 ± 2.08	34.80 ± 1.62
1	44.18 ± 3.27	37.57 ± 0.80	25.35 ± 0.64	46.43 ± 1.52	41.51 ± 3.83	30.44 ± 0.29	52.70 ± 1.17	47.93 ± 1.60	40.83 ± 0.88	43.65 ± 1.77	41.39 ± 1.20
2	51.85 ± 1.05	42.58 ± 0.86	35.94 ± 2.09	57.53 ± 1.89	47.21 ± 2.04	37.07 ± 1.23	64.56 ± 2.57	52.17 ± 1.74	47.84 ± 0.93	45.38 ± 1.84	45.06 ± 1.09
4	63.60 ± 4.18	48.24 ± 1.11	42.60 ± 0.96	69.26 ± 2.27	54.19 ± 2.56	43.84 ± 1.07	71.48 ± 1.22	56.79 ± 1.90	52.23 ± 0.50	51.02 ± 1.80	50.65 ± 0.92
8	82.45 ± 4.34	60.87 ± 1.29	50.20 ± 0.81	82.83 ± 4.59	61.75 ± 3.44	49.19 ±1.13	83.86 ±2.37	64.86 ±2.16	59.01 ±0.58	59.43 ±1.66	59.01 ±0.52
12	91.23 ±1.53	64.61 ±1.62	59.90 ±0.83	89.75 ±5.16	71.13 ±3.14	62.67 ±1.91	92.48 ±3.30	70.34 ±2.35	66.85 ±0.66	70.38 ±2.23	69.88 ±0.96
20	98.60 ±0.69	73.77 ±1.12	69.82 ±1.61	98.39 ±1.75	81.32 ±1.16	74.16 ±0.83	98.46 ±2.30	85.72 ±2.86	79.48 ±0.84	79.98 ±1.97	79.42 ±0.30
24	101.82 ±0.51	79.62 ±1.40	74.79 ±0.76	102.00 ±0.66	88.80 ±2.82	85.91 ±1.11	102.05 ±1.96	90.73 ±3.03	87.76 ±2.66	86.35 ±2.00	85.75 ±0.11
28	-	83.07 ±1.27	79.86 ±1.02	-	94.49 ±0.86	90.88 ±1.16	-	96.43 ±3.22	93.43 ±2.24	93.3 2±2.55	92.66 ±0.74
32	-	92.90 ±3.95	87.90 ±0.43	-	99.07 ±0.71	94.49 ±1.05	-	101.91 ±3.40	99.20 ±1.57	97.57 ±2.50	96.88 ±0.52
36	-	100.8 ±1.55	99.13 ±1.06	-	101.7 ±0.14	99.89±1.7 3	-	-	-	102.04 ±2.63	101.32 ±0.57

Table 6 Formula of optimized batch (B12)

Ingredients	Quantity
Asenapine maleate	30 mg
Compritol ATO 888	600 mg
Iso-propyl alcohol	10 ml
Poloxamer 188	1.03%
Deionized water	30 ml

Table 7 *In vitro* drug release data of optimized batch

Time (hour)	% Cumulative Drug Release (n=3) (Mean ± SD)
0	0
0.5	21.34±2.18
1	25.36±2.25
2	37.20±1.87
4	49.00±1.52
8	56.83±1.11
12	65.92±0.29
20	72.68±1.27
24	81.47±1.17
28	87.26±1.02
32	92.37±1.47
36	98.34±1.57

Table 8 Release kinetic study of optimized batch

Batch	Higuchi kinetic R^2	Zero order Kinetic R^2	First order Kinetic R^2	Hixon-crowell R^2	K-Peppas	
					R^2	n value
B12	0.9688	0.9286	0.7528	0.9425	0.9841	0.3663

Table 9 Results of optimized batch B12 for response variable

Response variables	Constrains	Predicted value	Experimental value
Y_1 = Particle size	250-350 nm	319.988 nm	326.1 ± 3.27 nm
Y_2 = % EE	25– 65 %	54.76 %	53.13 ± 1.77 %

Drug content

The drug content of ASM-loaded SLNs is tabulated. The drug content was found to be in the range of100 ± 5% indicating that ASM was uniformly distributed in nanoparticle dispersion and there was no loss or degradation of ASM during the preparation of SLNs.

Data analysis of Y_1 (particle size)

The mean particle size and PDI results of all the eleven batches (B1-B11) of Asenapine maleate loaded SLNs are tabulated. The mean particle sizes of batches B1-B11 were found in the range of 240 to 400 nm and the PDI was in the range of 0.266 to 0.482. The results indicated a profound effect of amount of Compritol ATO 888 and % of Poloxamer 188 on the particle size. The response (Y_1) obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation,

$$Y_1 = +303.37 + 21.12\ A - 51.20\ B - 13.23\ AB - 3.77\ A^2 + 1.78\ B^2 \quad (3)$$

The wide range of coefficient value shows in the above equation. The model coefficients estimated by multiple linear regression for particle size.

The regression coefficients having P value < 0.05 are highly significant. From Table 4, it is clear that the terms A^2& B^2 having P value > 0.05, are insignificant in contributing to prediction of particle size. The reduced equation can now be written as,

$$Y_1 = +303.37 + 21.12\ A - 51.20\ B - 13.23\ AB \quad (4)$$

The two independent variables A (amount of Compritol ATO 888) & B (amount of Poloxamer 188) as well as the interaction term (AB) was found to be significant (P < 0.05) in affecting Y_1. The positive co-efficient value for independent variable A (+21.12) indicated positive effect on dependent variable Y_1 whereas negative coefficient for independent variable B (-51.20) and interaction term AB (-13.23) indicated negative effect on dependent variable Y_1.

From the ANOVA data, the Fcal value was found to be 2568.37, which is more than the F tab value of 2.51, indicating that the model was significant. The P value is < 0.05 for all the response factors indicating that the models are significant.

Batches B1, B2 and B3 contained 300, 450 and 600 mg of Compritol ATO 888 whereas % of surfactant was constant at 0.5%. The particle sizes of the batches B1, B2 & B3 were 326.7 nm, 341.4 nm and 393.4 nm respectively. The particle size was influenced by lipid concentration. Increasing the lipid content from 300 mg, 450 mg to 600 mg resulted in a subsequent increase in particle size. A similar trend of particle size was observed in batches B4 to B6 and B7 to B9, which contained increasing amounts of lipid from 300 mg to 600 mg, whereas the surfactant concentration was constant at 1.0%. and 1.5% respectively. The efficiency of homogenization decreases due to a higher viscosity

of the sample at higher lipid contents, resulting in larger particles. Also, at high lipid contents increases the probability of particle contact and an aggregation[6&33].

Batches B1, B4 and B7 contained 0.5%, 1% and 1.5% of Poloxamer 188 respectively, whereas the amount of Compritol ATO 888 was constant at 300 mg. The particle sizes of the batches B1, B4& B7 were 326.7 nm, 269.0 nm and 243.3 nm respectively. The results clearly showed that there was a gradual decrease in particle size with an increase in surfactant concentration from 0.5, 1 to 1.5 % (w/w) ($p<0.05$). This decrease in size at high surfactant concentrations might be due to effective reduction in interfacial tension between the aqueous and lipid phases leading to the formation of emulsion droplets of smaller size[40]. Higher surfactant concentrations effectively stabilized the particles by forming a steric barrier on the particle surface and thereby protect smaller particles and prevent their coalescence into bigger ones.

Amongst all the batches, batch B7 containing lowest amount of lipid (600 mg) and highest amount of surfactant (1.5%), exhibited smallest particle size (243.3 nm). This can be explained by the surfactant-induced reduction in surface tension between aqueous phase and organic phase and stabilization of the newly generated surfaces which prevents particle aggregation.

The PDI is an important parameter that governs the physical stability of SLNs dispersion and should be as low as possible for the long term stability of SLNs dispersion. The PDI defined as dispersion homogeneity, has the range of 0 to 1. Values close to 1 indicate heterogeneity and those less than 0.5 indicate homogeneity. The PDI value of all formulation was found in the range of 0.066 to 0.281 which was less than 0.5, indicating their homogeneity and narrow particle size distribution[36].

Zeta potential provides information related to the storage stability of colloidal dispersions. The zeta potential values of all the batches ranged between -5 to -30 mV, indicating a relatively good stability and dispersion quality[40]. The surfactant concentration affected the charge on the particle. It was seen that as the surfactant concentration was increased from 0.5 to 1.5%, the zeta potential value became more negative[39]reported a similar finding, upon increasing Tween 80 concentrations from 0.5 to 1 %, which was attributed to the formation of denser surfactant film. Poloxamer 188 being non-ionic surfactant succeeded in the producing relatively stable dispersions. Although, non-ionic surfactant could not ionize into charging group like ionic ones, but still demonstrated its zeta potential. 1adsorption of emulsifier molecule on the charge in water, it was absorbed to the emulsifier layer of particle/water interface and electric double layer similar to ionic was formed[41]reported that poloxamer 188was found to be one of the most effective non-ionic surfactants to avoid the formation of aggregates. Poloxamer 188 can provide additional steric stabilization of particles; so we can expect combined electrostatic and steric stabilization of SLN formulations[30].

Data analysis of Y_2 (%EE)

The ultracentrifugation method was used to determine %EE of SLNs. At 25% to 55% the %EE was varied. The results clearly indicated that Y_2 is strongly affected by the amount of lipid and concentration of surfactant selected (Table 3) for the study. The response (Y_2) obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation.

$$Y_2= +45.15 + 11.76\ A- 4.10B- 0.43AB- 1.57A^2-0.087B^2$$

The wide range of coefficient values was showed by the above equation. The model coefficients estimated by multiple linear regression for %EE. The regression coefficients having P value < 0.05 are highly significant. The terms AB & B^2having P value > 0.05 were insignificant in contributing to prediction of particle size. The reduced equation can now be written as,

$$Y_2= +45.15 + 11.76\ A- 4.10B- 1.57A^2$$

The two independent variables A (amount of Compritol ATO 888) & B (amount of Poloxamer 188) was found to be significant ($P < 0.05$) in affecting Y_2. The positive co-efficient value for independent variable A (+11.76) indicated positive effect on dependent variable Y_2. While negative coefficient for independent variable B (-4.10) indicated negative effect on dependent variable Y_2.

From the ANOVA data, the F cal value was found to be 58.56, which is more than the F tab value of 2.51, indicating that the model was significant. Batches B1, B2 and B3 contained 300, 450 and 600 mg of Compritol ATO 888 respectively whereas amount of surfactant was constant (0.5%). The %EE of the batches B1, B2 & B3 were 34.90%, 49.49% and 59.97% respectively. Similar trend was observed in batches- B4 to B6 and B7 to B9. Increase in amount of lipid led to increase in %EE. This could be due to availability of more lipid molecules, when a higher lipid matrix is used. The higher lipid matrix provides an increased accommodation of lipophilic drugs due to the long chain fatty acids attached to triglyceride. Our results are in agreement with findings[34].

It was also evident that increasing the amount of surfactant from0.5, 1to 1.5 % (w/w) at a constant amount of lipid, resulted in a gradual significant decrease in the % EE of the ASM-loaded-SLNs ($p<0.05$). Partition phenomenon explained the decrease in %EE. High surfactant level in the external phase might increase the partition of drug from internal to external phase of the medium. This increased partition is due to the increased solubilization of the drug in the external aqueous phase so more drug can disperse and dissolve in it[35].

Contour plots and response surface analysis

Two-dimensional contour plots and 3-D response surface plots for variables Y_1 (particle size) are shown in Figures 1 & 2 respectively. Similarly, two-dimensional contour plots and 3-D response surface plots for variables Y_2 (%). They are very useful to study the interaction effects, main effects and quadratic effects of the factors (independent variables) on the responses (dependent variables). These types of plots are helpful in portraying the study of the effects of two factors on the response at a time. Figures 1 & 2 reveal a decline in particle size with an increase in concentration of surfactant. Increase in amount of lipid led to an increase in particle size. The lowest particle size was reported with the highest amount of surfactant and the lowest amount of lipid. An increase in entrapment efficiency with an increase in concentration of lipid. Due to the highest amount of lipid, he highest entrapment efficiency was obtained. The desirability and counter plots were constructed and the optimized formulae were predicted using the constraints on the dependent variables. The desirability function was found to be near to 1 for the optimized formula indicating the suitability of the formulations. The batch B6 was matched with the composition of the optimized formulations. However, for the reconfirmation of result the experiment was repeated (Batch B12). The Batch B12was analyzed for particle size and %EE. The % relative error for particle size and %EE within the experimental and predicted values for batch B12 was found to be 3.7% and 2.8% respectively.

However, the values were found to be < 5 %and hence it confirmed the suitability of experimental design followed for this study.

In vitro drug release study

The modified dialysis method used for study In vitro drug release of ASM-loaded-SLNs. The initial rapid drug release was around 25-50% within 1 hr in all batches. The initial rapid drug release may be due to the presence of free ASM in the external phase and on the surface of the nanoparticles. The initial burst release will be beneficial in achieving the required drug levels in the plasma. Following 32 h showed >90% cumulative percent drug release representing release of drug from the nanoparticles, vital for providing sustained drug levels in the blood. The lipophilic nature of ASM could be the reason for sustained release of the drug from internal lipid phase after initial burst release. The rate of drug release also influenced by particle size. Smaller nanoparticles lead to a shorter average diffusion path of the ASM entrapped and lead to faster rate of release of the entrapped drug compared to bigger size NP. The larger nanoparticles could sustain the release of the drug up to 36 hour. The results obtained are in accordance with the findings of several authors who claimed that the particle size differences are a significant factor for drug release rate kinetics in nanoparticulate drug delivery systems[35&36]. The concentration of lipid and surfactant were also affect the rate of drug release. An inverse relationship was found between the lipid concentration and drug release rate be due to the higher concentration of drug presence in the inner core. The decrease in release profile observed can be credited to the higher lipid content encapsulating the drug thus reducing drug partition in the outer phase and consequently its release in the receiver media. The release profiles of SLNs resemble the drug enriched core model[38]. In such a model, the drug enriched core is surrounded by a practically drug-free lipid shell. The drug has a sustained release profile due to increased diffusional distance and hindering effects by the surrounding solid lipid shell. As the surfactant concentration increased, rapid drug release rate was obtained. This could be explained by the partitioning effects of the drug between the melted liquid. Drug partitions from the liquid oil phase to the aqueous water phase shown during the particle production. The higher the surfactant concentration, greater is the solubility of the drug in the aqueous phase so the amount of drug in the outer shell will be increased and released in a relatively rapid way[39].

Optimization

The optimum formulation was selected based on the criteria of attaining the constraints of variables response. Upon trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search was done. The composition of formulation contain 600 mg Compritol ATO 888 (lipid) and 1.03 % of Poloxamer 188 (surfactant) were found to fulfill an optimum formulation (Batch B12)& In vitro drug release data.

Drug content of the optimized batch was found to be 99.56 % ± 1.86. The drug release of ASM-loaded-SLNs (Batch B12) (table 8) was fitted to different kinetic models to understand the drug release mechanism and kinetics. The release was fitted to both the zero order and first order models. R^2 values for zero order and first order models were found to be 0.93 and 0.75 respectively, indicating that the release followed zero order release kinetics. The drug release data was fitted to Korsmeyer-Peppas model to determine the value of diffusion exponent (n). The value of n for a spherical system is< 0.5 for Fickian release; 0.5 < n < 1 indicates non-Fickian release; n > 1 indicates super case II release. The n value for Batch B12 was less than 0.5 therefore the release mechanism is said to follow Fickian diffusion kinetics. It can be concluded that the release of Asenapine maleate from the SLNs follows first order kinetics and mechanism of drug release is Fickian.

The particle size of the optimized batch was found to be 326.1 ± 3.27 nm with the PDI value 0.486 ± 0.08. Smaller PDI value suggests homogeneity and narrow size distribution (Table 9). The zeta potential of the formulation was found to be -30.4 ± (-0.92) mV, which indicate the stability SLNs dispersion.

XRD studies

XRD patterns of ASM, Compritol ATO 888, physical mixture of the ASM and Compritol ATO 888 and lyophilized ASM-loaded-SLNs are shown in the Figures 7, 8, 9 and 10 respectively. XRD pattern of ASM exhibited sharp peaks at 2θ of8.9, 20.7, 21.6, 25.1and 28.6indicating the crystalline nature of the drug. The XRD pattern of Compritol ATO 888 was showing peak at about 20.9 and 23.9 22θ indicating the crystalline nature of the lipid. All the major peaks of drug and lipid were present in the physical mixture of the ASM and Compritol ATO 888 but with reduced intensity. The peaks of ASM were absent in the XRD pattern of SLNs, indicating that the drug lost its crystalline nature.

DSC studies

DSC thermograms of ASM, Compritol ATO 888), physical mixture of the ASM and Compritol ATO 888 and lyophilized ASM-loaded-SLNs were recorded. The DSC thermograms of ASM (Figure 11) showed a sharp endothermic peak at 144.67°C, corresponding to its melting point, indicating its crystalline nature. Figure 12 exhibited a sharp endothermic event at 68.96°C, which can be attributed to the melting point of Compritol ATO 888. The DSC thermogram (Figure 13) of physical mixture of ASM and Compritol ATO 888 (1:1) exhibited both component peaks and no significant shift in the position of individual endothermic peaks, indicating absence of any chemical interaction between ASM and Compritol ATO 888. DSC thermogram of ASM-loaded-SLNs (Figure 14) exhibited an endothermic peak at 66.22 °C for Compritol ATO 888, but did not show the melting peak of ASM, indicating the conversion of crystalline ASM to the amorphous form which could be attributed to complete dissolution of the drug in the molten lipid matrix.

Accelerated stability studies

Stability studies were carried out for the optimized formulation (Batch B12) in aqueous dispersion and lyophilized state over a period of 3 months at 4 °C, 25 °C ± 2 °C/60% ± 5% RH and 40 °C ± 2 °C/75% ± 5% RH. Particle size and assay were employed as a characterization tool to study the effect of different storage conditions. Figures 15 & 16 exhibited the effect of various storage conditions on the mean particle size of optimized batch (Batch B12) initially and after 1, 2 and 3 months.

There was no significant change (P > 0.05) in the mean particle size of SLNs in lyophilized state at 4 °C up to 3 months and in the aqueous dispersion state for 2 months. The mean particle size of SLNs in aqueous dispersion increased from initial 262 ± 2.6 nm to 289.1 ± 2.3 nm at the end of 3 M.

At 25 °C ± 2 °C/60% ± 5% RH, there was no significant change (P > 0.05) in the mean particle size up to 2 months in the lyophilized state and up to 1 month in the aqueous dispersion state. At the end of 3 M, the particle size of nanoparticles in lyophilized state was increased from 326.1 ± 3.27 nm to

359.6±4.7 nm. In case of aqueous dispersion state, the particle size changed from 262 ± 2.6nm to 276.3 ± 5.9nm and 301.4 ± 2.8nm at the end of 2 and 3 M respectively.

At 40 °C ± 5 °C/75% ± 2% RH, there was no significant change (P > 0.05) in the mean particle size up to 1 M in the lyophilized state, whereas the particle size increased from 326.1 ± 3.27 nm to 344.3 ± 4.4 nm and 392.7 ± 6.9 nm respectively. In case of aqueous dispersion state, ASM-loaded-SLNs were found to be aggregated. The particle size increased from 262 ± 2.6 nm to 344.8 ± 3.1nm, 441 ± 5.2 nm and 478.5 ± 4.0 nm at the end of 1, 2 and 3 M. At higher temperature, the NPs were not stable and a large increase in particle size was observed. Higher temperature reduces the micro viscosity of the emulsifier and encourages destabilisation of the system. Higher temperatures also increase the kinetic energy of the SLNs which is enough to overcome the electrostatic repulsion and form agglomerates. The results are in conformation with findings[40].

There was no significant change (P>0.05) in the drug content for ASM-loaded-SLNs in the lyophilized state at 4°C up to 3 M and for 2M in aqueous dispersion state. The drug content decreased from 99.56% to 95.41% at the 3 M in aqueous dispersion state.

At 25°C ± 5°C/60% ± 2% RH, batch B12 was stable in lyophilized state up to 2M after which the drug content was reduced to 93.7% in the third month. In the aqueous dispersion state, there was no significant change (P>0.05) in the drug content up to 1 M. The drug content decreased from 99.56% to 93.63 % and 89.45% at the end of 2 and 3 M respectively.

At 40°C ± 5°C/75% ± 2% RH, there was no significant change (P > 0.05) in the drug content up to 1 M in the lyophilized state. The drug content decreased from 99.56% to 86.33% and 79.90% at the end of 2 and 3 M respectively. Drug content was reduced to 84%, 79.27% and 74.08% at the end of 1, 2 and 3 M respectively in aqueous dispersion state.

It was concluded that it was best to store SLNs in lyophilized state at 4 °C, where they did not show any difference in particle size and drug content. Figure 15-16 confirms that lyophilization could be a suitable technique to reduce risk of particle size enlargement and particles aggregation, thereby providing stability to NPs.

Transmission electron microscopy

TEM was performed to obtain additional information on particle morphology and aggregation phenomena.
The NPs required a smooth surface, which may contribute to release of the drug in a sustained manner.
No aggregation of the particles was observed.

CONCLUSION

The ASM-loaded SLNs were successfully formulated using Compritol ATO 888 as carrier lipid and Poloxamer 188 as surfactant using High pressure homogenization technique. The optimization of amount of lipid and % surfactant in the SLNs formulation was carried out using 3^2 full factorial designs. The developed SLNs exhibited controlled drug release upto a period of 36 h. The developed formulation was found to be stable with no significant change in particle size, and drug content. *In vivo* study of optimized ASM-loaded SLNs confirmed the controlled release of drug from the formulation in comparison to oral drug solution.The biphasic appearance of drug in plasma confirms the drug absorption via enteral and lymphatic route. There was significant increase in % relative bioavailability in comparison to oral drug solution. The SLNs due to their size and lipophilic characteristics may be useful in avoiding the first pass metabolism. The ASM-loaded SLNs may provide a better bioavailability, reduction in dose, dosing frequency, dose related side effects and better control of the disease.

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