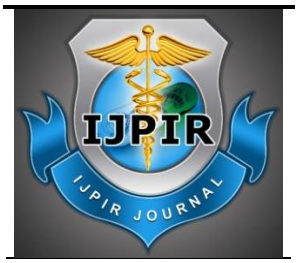
Research Article



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Design characterization of atomoxetine loaded solid lipid nanoparticle

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ABSTRACT

Nano-materials with excellent biodegradability and biocompatibility are considered to be the best vehicles for drug delivery systems in biomedical applications. Currently, scientists and researchers are focused on discovering new methods/routes to control the pharmacokinetics (ADME), pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and drug efficacy of drugs. These new strategies are often called novel drug delivery systems (NDDS) and are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To formulate and characterize the Atomoxetine loaded Solid lipid nanoparticle by solvent evaporation method. To formulate the solid lipid nanoparticles of Atomoxetine. To study the drug-polymer compatibility by FTIR and to characterize the solid lipid nanoparticles for particle size, Entrapment Efficiency, zetapotential. To study the drug release of SLN formulations and the drug release kinetics of SLN, and stability of optimized SLN formulation.

The In-vitro drug release for all nine formulations was evaluated by the dialysis bag diffusion technique. In-vitro drug release for the optimized FSLNA7. Showed the best result by releasing atomoxetine 99.01% in 12hr. The drug release kinetics was calculated by fitting the dissolution data in various equation the results in Table No.9 depicting the best formulation is following zero order kinetics results.

In the present research work the solid lipid nanoparticle of Atomoxetine was formulated by solvent evaporation method, The ingredient s like lipid (stearic acid), surfactant (tween 20) and co-surfactant (PG) were selected for formulation using methanol as solvent. A solution of Atomoxetine in phosphate buffer pH 7.4 was scanned in UV range between 200 to 350 nm (LabIndiaUV 3200 spectrophotometer, India). Atomoxetine showed maximum absorbance at 274 nm in phosphate buffer pH7.4. FT-IR studies suggested that there is no interaction between the drug and the polymer. It was concluded that the Atomoxetine loaded Solid lipid nanoparticle FSLNA7 is the best formulation can be considered for animal study.

Keywords: nanostructured lipid carrier (NLC), FSLNA7, Formulation, FTIR,

INTRODUCTION

With the development of technology in the last two decades, the particle size of materials ranges from the micro- to nano-scale. The reduction in the particle size of materials at the nanometer scale increases their overall surface area by several orders of magnitude. Particles with a size in the range of 1 nm to 1000 nm are known as nanoparticles.

However, nano-materials with excellent biodegradability and biocompatibility are considered to be the best vehicles for drug delivery systems in biomedical applications. Currently, scientists and researchers are focused on discovering new

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methods/routes to control the pharmacokinetics (ADME), pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and drug efficacy of drugs. These new strategies are often called novel drug delivery systems (NDDS) and are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. Some of the different approaches for novel drug delivery include transdermal patches, sustained and controlled release by polymeric and magnetic control, liposomes, hydrogels, implants, microspheres, erythrocytes, and nanoparticles. Nanoparticular drug delivery systems are a successful approach in the treatment of chronic human diseases, which have excellent function in satisfying the biopharmaceutical and pharmacological considerations. The emergence of nanotechnology and the growing capabilities of functional proteomics, genomics, and bioinformatics combined with combinatorial chemistry have driven scientists to become more enthusiastic to express their technical expertise to discover, invent and explore novel approaches for drug delivery systems through new techniques. Novel drug delivery systems remain the foundation to deliver drugs having complications that cannot be minimized by conventional drug delivery systems, where the therapeutic effectiveness of drugs depends on their pharmacokinetics and site of administration. Pharmacokinetics are also based on physico-chemical properties such as solubility, crystallinity, toxicity, and HLB value. After understanding the biopharmaceutics and pharmacokinetics, the administration route, absorptive surface area, and transportation of drugs in the body are the key points for their absorption and distribution. Furthermore, metabolism and elimination depend on the aforementioned properties.[1] The formulation design has a major impact on the effective delivery of the active pharmaceutical ingredient (API), and thus all the above parameters are crucial challenges. Drugs based on the HLB scale are categorized into two classes, hydrophilic and lipophilic molecules. Lipophilic molecules exhibit very poor solubility, and depending on this, they produce a great challenge to design safe, efficacious, and cost-effective drug delivery systems and have been a source of frustration for pharmaceutical scientists.[2]

Lipophilic molecules allow the design of formulations for hydrophobic drug molecules, and despite all the problems confronted by pharmaceutical scientists, the current solid lipid nanoparticles are the result of their great effort. Traditionally, lipid-based novel drug delivery systems have focused on the delivery of lipophilic molecules, but recently, lipoid drug delivery systems have received attention due to their inherent properties such as biocompatibility, self-assembly capabilities, ability to cross the blood brain barrier, particle size variability and finally cost effectiveness, making lipid-based delivery systems much more attractive.

MATERIALS

Table 1: Materials Used in Formulation

S. NO	MATERIALS NAME	COMPANY
1	Atomoxetine HCI	Dr. Reddy's Laboratories., Hyderabad, India.
2	Propylene Glycol	Spectrum Chemicals Ltd
3	Stearic acid	Universal scientific
4	Tween 20	SD Fine Chemicals Mumbai
5	Ethanol	Rankem
6	Methanol	Rankem

Table 2: Equipments

S. NO	EQUIPMENT NAME	MAKE
1	UV-Visible spectrophotometer	LabIndia UV3200
2	Refrigerator	Whirl Pool
3	PH Meter	LabIndia DS 2000
4	FT-IR spectrophotometer	Bruker Alpha T - 1020
5	Scanning electron microscope	Jeol Ltd Japan
6	Electronic digital balance	Shimadzu AX200 and ATX 224
7	Magnetic stirrer	2MCH Remi
8	Probe Sonicator	Ultrasonic Processor VCX 750
9	Vortex mixer	LabIndia
10	Stability Chamber	Cintex

Preformulation Studies

Colour :white to practically white in colour.
Solubility :Freely soluble in water and Methanol.
Melting Point : 161-165^0 C

Identification :A solution of Atomoxetine in phosphate buffer 7.4 was scanned in UV range between 200 to 350nm.
Calibration Curve: Accurately weighed Atomoxetine(50mg) was dissolved in phosphate buffer and serial dilutions are made inorder to obtain 5,10,15,20,25,30 µg/ml respectively.

The absorbance of these was determined in a UV spectro-photometer at 274 nm.

IR Spectrum Of pure Drug: IR spectrum scan was performed between the range of 4000 to 400cm $^{-}$

Preparation of Atomoxetine loaded SLN[11, 12]

SLNs were prepared using the emulsification solvent evaporation technique. Briefly, drug and lipid were dis-solved in methanol. This organic phase was added drop wise to an aqueous solution containing surface active agent(s) and Co surfactant. The obtained pre-emulsion was subsequently subjected to ultrasonication using probe sonicator (Ultrasonic processor model VCX 750) to decrease the globules size to the required nanometer range. The formed emulsion was stirred at the room temperature using a magnetic stirrer at 400 rpm to allow the organic solvent to evaporate and SLNs to be formed.

Table 3: Formulation Table for Atomoxetine loaded SLN

Ingredients	FSLN A1	FSLN A2	FSLN A3	FSLN A4	FSLN A5	FSLN A6	FSLN A7	FSLN A8	FSLN A9
Atomoxetine (mg)	10	10	10	10	10	10	10	10	10
Stearic Acid(mg)	20	30	40	20	30	40	20	30	40
Tween 20 (ml)	5	5	5	10	10	10	15	15	15
Propylene Glycol(ml)	5	5	5	5	5	5	5	5	5
Methanol (ml)	20	20	20	20	20	20	20	20	20

Characterization of SLN

Particle size

Average vesicle size was measured using a Malvern particle size analyzer, (Malvern Master sizer 2000 instruments Ltd., UK). 5 ml of each nanosomal suspension was dispersed in 500 ml of double distilled water under gentle stirring (600 rpm) in a glass beaker. All measurements were means of triplicate (mean ± SE).

Entrapment efficiency[58]

The entrapment efficiency (EE) of the SLNs was determined by indirect method wherein the amount of unentrapped drug in the aqueous surfactant solution i.e., supernatant after centrifugation at 22,000 rpm for 45 min was determined, against the total amount of drug added to the formulation. The supernatant was diluted appropriately with 0.1 N HCl and analyzed by using U.V Spectrophotometer at Particular wavelength nm.

$$\% \text{ Entrapment Efficiency} = \frac{\text{Amount of Drug added initially} - \text{Amount of Drug present in the supernatant}}{\text{Amount of Drug Added Initially}} \times 100$$

Zeta potential

The zeta potential of a particle represents the overall charge of the particle and stability of the formulation. Zeta potential measurement was carried out using Zeta Sizer Nano-ZS90, Malvern Instrument Ltd.,UK by differential light scattering (DLS) technique. Nanoparticle samples are redispersed in Milli-Q water. All measurements were carried out in triplicates at 25 °C.

In vitro release studies and release kinetics[32]

In vitro profile of drug loaded SLN formulation was carried using dialysis bag, using phosphate buffer 7.4. Dialysis membrane having pore size 2.4nm and a molecular weight cut off of 1200 to 14,000Da. Membrane was soaked in a distilled water for 15mins before using. The formulation 2mg equivalent was placed inside the dialysis bag and immersed in a beaker containing 200 ml phosphate buffer pH7.4and the system was maintained 37· C ± 2 · C with 100 RPM, at predetermined time interval, aliquots of the samples was withdrawn and fresh medium was replaced. The samples was analysed spectrophotometrically by measuring the absorbance at 274nm.

Release kinetics

The evaluation of the mechanism of drug release and its kinetics was performed using mathematical models. The models that best fit the data were selected based on the correlation coefficient(R) value in various models. The mathematical models used were Korsmeyer-Peppas model, Hixson Crowell model, Higuchi model, First order and Zero order release model.

Morphological studies by scanning electron microscopy (SEM)

The surface morphology of freeze dried SLN powder without the addition of mannitol was observed using SEM (JEOL, JSM 50A, Tokyo, Japan). An appropriate amount of SLNs was mounted on the metal(aluminium) stubs, using double sided adhesive tape and fractured with a razor blade. Gold/palladium sputter coating for 120 s at 14 mA under argon atmosphere was performed for secondary electron emissive SEM. The morphology was observed at an acceleration voltage of 15 KV

Stability Study[20]

Stability Studies Selected formulations were stored at different storage conditions at elevated temperatures such as 25^0 C±2^0C/60%±5%RH, and 40^0 C± 2^0C /75%±5% RH at for 180days. The samples were withdrawn at intervals of 30 days and checked for physical changes, Particle size and EE%.

RESULT AND DISCUSSION

Table 4: Description of Drug

S. No	Parameter	Atomoxetine
1	Colour	White to practically white solid
2	Solubility	Soluble in water
3	Melting Point	161-165^0 C
4	Molecular Formula	$C_{17}H_{22}ClNO$
5	Molecular weight	291.82
6	Odour	No
7	Hygroscopicity	Non hygroscopic

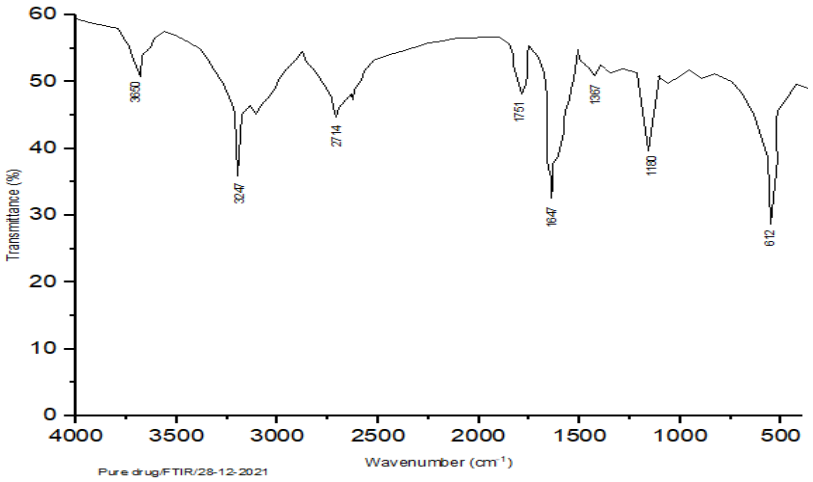

Fig 1: FTIR of Pure Atomoxetine

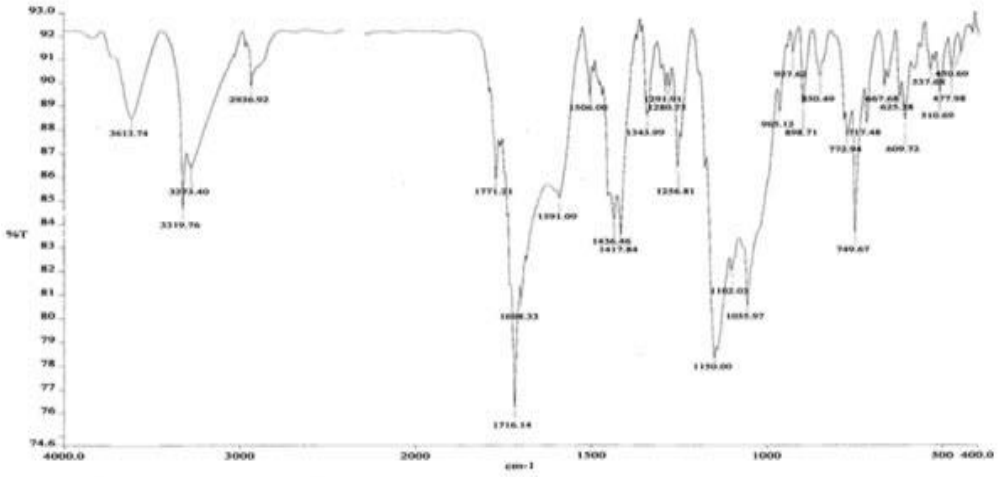

Fig 2: FTIR of Drug with Polymer

Table 5: Formulation Table for Atomoxetine loaded SLN

Ingredients	FSLN A1	FSLN A2	FSLN A3	FSLN A4	FSLN A5	FSLN A6	FSLN A7	FSLN A8	FSLN A9
Atomoxetine (mg)	10	10	10	10	10	10	10	10	10
Stearic Acid (mg)	20	30	40	20	30	40	20	30	40
Tween 20 (ml)	5	5	5	10	10	10	15	15	15
Propylene Glycol (ml)	5	5	5	5	5	5	5	5	5
Methanol (ml)	20	20	20	20	20	20	20	20	20

Table 6: Characterisation of Atomoxetine loaded SLN (FSLNA)

Formulation	Particle size(nm)	EE%	Zeta potential(mV)
FSLNA1	217±6.24	70.17±1.01	-21
FSLNA2	270±8.21	72.8±2.04	-23
FSLNA3	323±11.04	81.23±1.32	-20
FSLNA4	243±10.45	59.37±2.36	-23
FSLNA5	283±11.35	76.93±2.14	-28
FSLNA6	301±12.01	61.62±1.52	-20
FSLNA7	101±8.14	85.59±1.07	-28
FSLNA8	156±9..47	60.82±1.47	-25
FSLNA9	207±10.24	59.47±1.78	-30

(values=Mean ±SD , n=3)

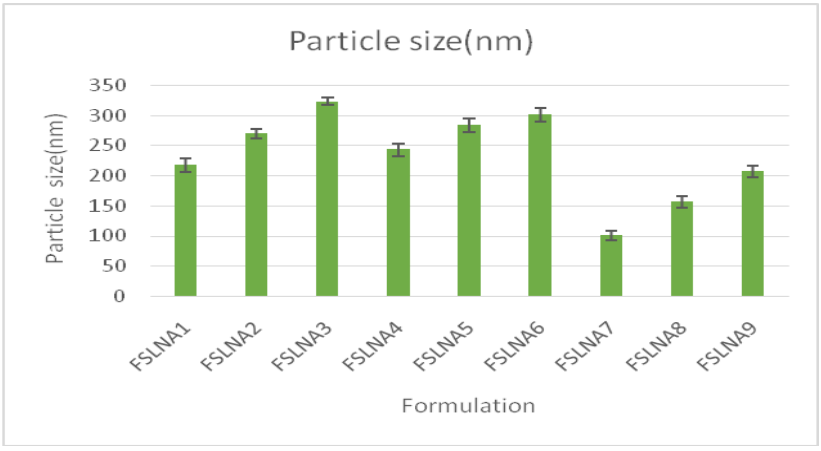

Fig 3: Particle size of Atomoxetine loaded SLN(FSLNA1-FSLNA9)

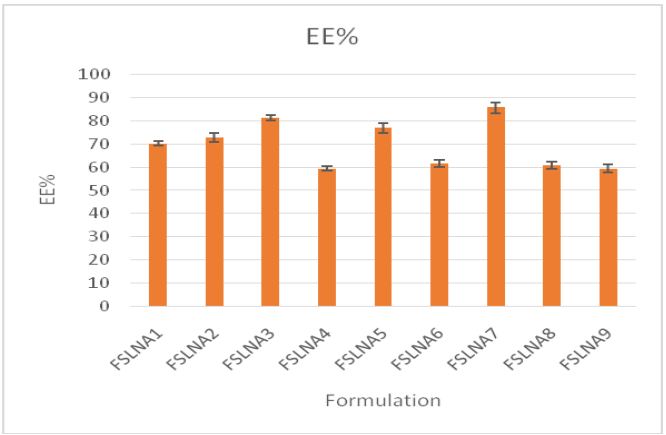

Fig 4: Entrapment Efficiency% of Atomoxetine loaded SLN(FSLNA1-FSLNA9)

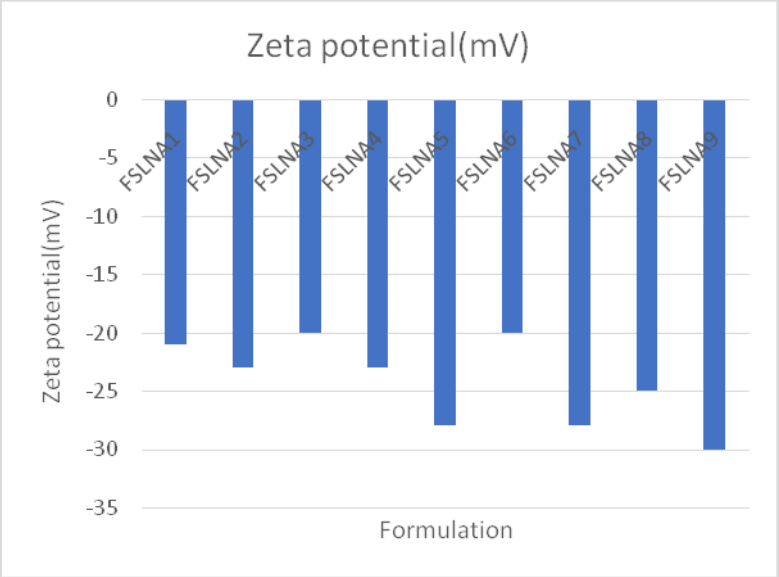

Fig 5: Zeta potential of Atomoxetine loaded SLN (FSLNA1-FSLNA9)

Table 7: Invitro Drug Release

Time (hr)	FSLN A1	FSLN A2	FSLN A3	FSLN A4	FSLN A5	FSLN A6	FSLN A7	FSLN A8	FSLN A9
	In-vitro Release %								
0	0	0	0	0	0	0	0	0	0
1	30.26	28.13	11.35	15.24	21.36	8.76	12.14	22.35	20.26
2	51.33	48.36	32.14	28.13	41.33	12.14	31.25	42.14	61.33
4	79.24	68.24	45.78	50.12	56.28	31.25	46.14	45.78	89.24
6	98.31	85.44	68.14	67.32	61.25	46.14	58.34	58.14	98.44
8	-	97.14	79.14	73.19	80.32	58.34	79.77	89.14	-
10	-	-	99.21	78.13	99.14	79.77	88.76	98.21	-
12	-	-	-	82.13	-	88.76	99.01		-

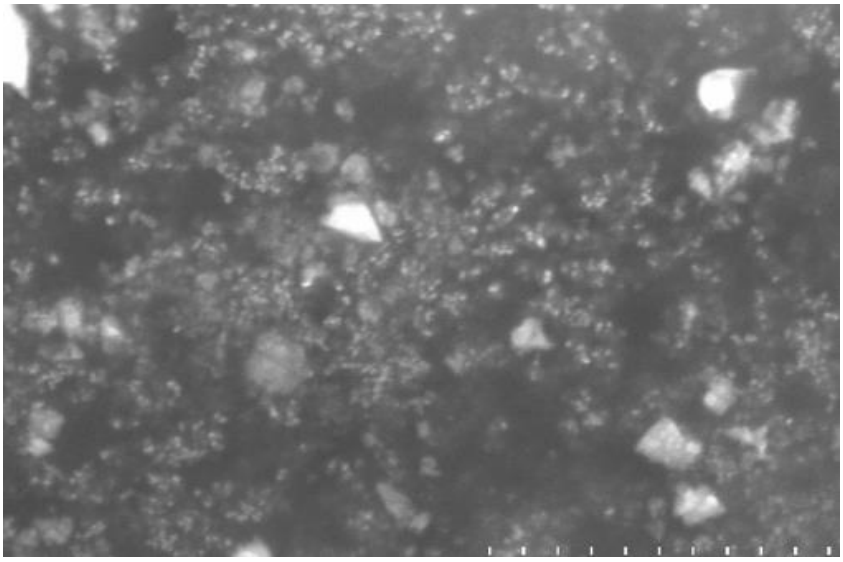

Fig 6: Scanning electron microscope of Atomoxetine SLN

In Vitro drug release study

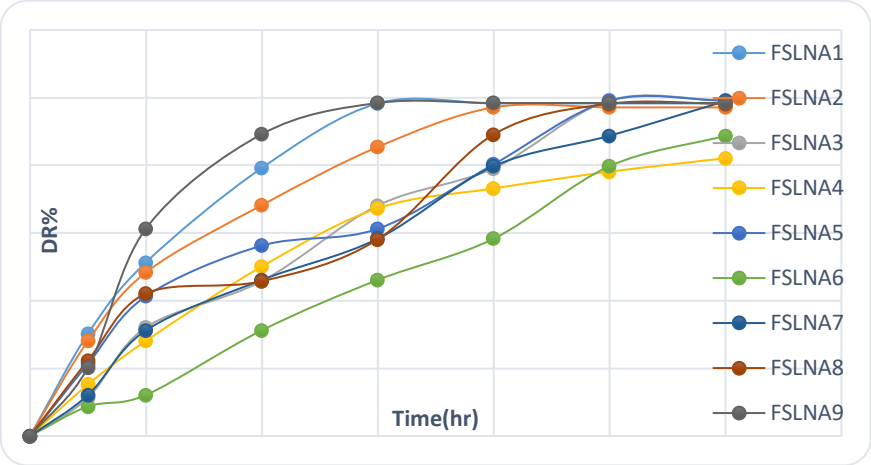

Fig 7: In vitro drug release study of Atomoxetine SLN(FSLNA1 to FSLNA9)

Table 8: Release kinetic parameters of Atomoxetine SLN

Formulation	Zero order	First order	Higuchi	Korsmeyer Peppas	N-values
FSLNA1	0.998	0.874	0.991	0.994	0.807
FSLNA2	0.947	0.894	0.947	0.944	0.718
FSLNA3	0.987	0.974	0.984	0.981	0.845
FSLNA4	0.997	0.875	0.991	0.998	0.866
FSLNA5	0.954	0.861	0.956	0.953	0.874
FSLNA6	0.965	0.876	0.968	0.968	0.841
FSLNA7	0.999	0.843	0.907	0.994	0.798
FSLNA8	0.968	0.872	0.975	0.960	0.872
FSLNA9	0.987	0.853	0.981	0.988	0.741

Table 9: Stability Results for best formula FSLNA7

Days	25^0 C±2^0 C/60%±5%RH			40^0 C± 2^0C /75%±5% RH		
	Appearance	**Particle size**	**EE%**	**Appearance**	**Particle size**	**EE%**
0	Clear	101±8.14nm	85.59±1.07	Clear	101±8.14nm	85.59±1.07
30	Clear	101±8.18nm	85.58±1.17	Clear	101±8.08nm	85.78±1.17
60	Clear	101±8.24nm	85.42±1.27	Clear	101±8.34nm	85.32±1.26
90	Clear	102±8.04nm	85.39±1.27	Clear	101±7.04nm	84.39±1.29
180	Clear	102±8.44nm	84.89±1.17	Clear	102±7.14nm	84.29±1.07

The IR spectrum of the pure drug

Atomoxetine exhibited its characteristic absorption bands (3652 N-H stretching) in the IR region. The FT-IR spectrum of pure drug and FT-IR spectra of the polymers showed that there is a negligible difference in the position of characteristics of absorption bands of the functional groups of the drug. Thus, it is clear from FTIR study that there is no interaction of the drug with the polymer.

Characterization of Atomoxetine loaded SLN

Particle size

The mean particle size was observed in between range of 101± 8.14nm to 323 ±11.04nm. The least mean particle obtained for formulations FSLNA7 due to less content of lipid, while maximum particle size was obtained for FSLNA3.

Entrapment Efficiency%

The Entrapment efficiency in developed formulations produces higher load for all formulations. Entrapment efficiency was obtained in between the range of 59.37±2.36 to 85.59±1.07 .The highest entrapment efficiency was found for FSLNA7 due to less content of lipid and higher content of surfactant. Results of EE%.

Zetapotential

Zeta potential for all nine formulation obtained in between -20 to -30 mV.

Scanning electron microscope

A surface study of the freeze dried SLNs using SEM (Figure No.23) displayed aggregation and fusion of the particles, which could be attributed to the mechanical stress of ice crystals, that are formed during the freeze drying process, on the vesicles wall as previously mentioned.

In-vitro drug Release

The In-vitro drug release for all nine formulations was evaluated by the dialysis bag diffusion technique reported by Reddy and Murthy (2005) with slight modification, In-vitro drug release for the optimized FSLNA7. Showed the best result by releasing atomoxetine 99.01% in 12hr. The drug release kinetics was calculated by fitting the dissolution data in various equation the results in Table No.9 depicting the best formulation is following zero order kinetics

Stability Studies[20]

There was no significant changes in Physical appearance, mean particle size and entrapment efficiency was observed in selected FSLNA7 formulation after various time point and temperature condition which refers it as a stable formulation.

CONCLUSION

In the present research work the solid lipid nanoparticle of Atomoxetine was formulated by solvent evaporation method, The ingredient s like lipid (stearic acid), surfactant (tween 20) and co-surfactant(PG) were selected for formulation using methanol as solvent. A solution of Atomoxetine in phosphate buffer pH 7.4 was scanned in UV range between 200 to 350 nm (LabIndia UV 3200 spectrophotometer, India). Atomoxetine showed maximum absorbance at 274 nm in phosphate buffer pH7.4. FT-IR studies suggested that there is no interaction between the drug and the polymer. All the nine formulation prepared were evaluated for particle size, entrapment efficiency, zeta potential. The mean particle size was observed in between range of 101±8.14nm to 323±11.04nm. The least mean particle obtained for formulation FSLNA7. Entrapment efficiency was obtained in between the range of 59.37±2.36 to 85.59±1.07 .The highest entrapment efficiency was found for FSLNA7. Zeta potential for all nine formulation obtained in between -20 to -30 mV. A surface study of the freeze dried SLNs using SEM displayed aggregation and fusion of the particles, which could be attributed to the mechanical stress of ice crystals. In-vitro drug release for the optimized FSLNA7. Showed the best result by releasing Atomoxetine 99.01% in 12hr. There was no significant changes in Physical appearance, mean particle size and entrapment efficiency was observed in selected FSLNA7 formulation after various time point and temperature condition which refers it as a stable formulation. From above data it was concluded that the Atomoxetine loaded Solid lipid nanoparticle FSLNA7 is the best formulation can be considered for animal study.

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