Formulation & evaluation of aripiprazole solid lipid nanoparticles for brain targeting

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ABSTRACT

The objective of this research work was to formulate Aripiprazole solid lipid nanoparticles for brain targeting by surpassing the Blood brain barrier (BBB) and increasing the retention time in the brain for better therapy. The peaks obtained in the physical mixture were mostly identical with the peaks of the pure sample indicating their compatibility. The Solid lipid nanoparticles containing Aripiprazole was formulated by High speed homogenization technique using Ultra Turrax T25, IKA high speed homogenizer. When subjected to evaluation, it was observed that maximum entrapment efficiency was achieved with formulations F8 and F11. *In-vitro* diffusion studies of all the formulations followed first-order kinetics and ascertained Peppas mechanism. Based on the release profile and the entrapment efficiency, the formulations F8 & F11 were found to be the optimized .SEM analysis revealed that the particles were spherical in shape. The particle size range of the solid lipid nanoparticle formulations F8 and F11 were found to be 120nm to 400nm and 150nm to 520nm with the average particle size of 202.6nm and 263.9nm respectively. The zeta potential of the optimized formulations (F8 and F11) were found to be -28.9 mV & -33.1mV indicating the higher negative surface charge claiming to possess good stability. From the above results, it can be concluded that Aripiprazole Solid lipid nanoparticles could act as better formulation for the effective management of psychotic disorders.

Keywords: Aripiprazole, Nanoparticles, High speed homogenization technique**.**

INTRODUCTION

Psychosis is a mental disorder which results due to alterations in the monoamine neurotransmitters level in the Central nervous system. The manifestations include elusions, illusions and hallucinations (Richard E. Powers, 2008).Aripiprazole is a partial dopamine agonist of the third generation class of atypical antipsychotics with additional antidepressant properties that is primarily used in the treatment of schizophrenia, bipolar disorder, major depressive disorder and irritability associated with autism. Aripiprazole's antipsychotic activity is likely due to a combination of antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Antagonism at D2 receptors relieves positive symptoms while antagonism at 5HT2A receptors relieves negative symptoms of schizophrenia. The oral bioavailability is 87%.The main problem associated with this drug is its poor permeability into the brain due to efflux of drug by P-glycoprotein. Any formulation which could surpass this drug at Blood Brain Barrier could lead to better therapy (Megan et al, 2012)(Roger S, 2012).

Solid lipid nanoparticles are tiny colloidal carriers composed of biocompatible or biodegradable lipid matrix that is solid at body temperature, dispersed in aqueous surfactant solution and exhibit size range in between 50-1000nm. SLN's have distinct advantages over conventional dosage forms. They can encapsulate both lipophilic and hydrophilic drug moieties. SLN's are a useful strategy towards drug targeting particularly in case of brain. They are biocompatible, biodegradable and non-immunogenic .Aripiprazole SLN's can better target the brain and improves the therapeutic efficacy of the drug (Geddes J, 2000).

MATERIALS AND METHODS

Materials: Aripiprazole was procured as gift sample from Ranbaxy, Chennai; Glycerylmono-oleate (GMO) were purchased from Sigma Aldrich, Germany; Polaxamer-188, Triton X-100 and Tween 80 were purchased from Himedia Laboratories, Mumbai, India; and all other chemicals used throughout the study were of analytical grades.

The physicochemical compatibility between Aripiprazole, Surfactants (Span 20,Span 60,Span 80) and cholesterol used in the research were carried out by IR Spectral studies using Perkin Elmer Fourier transform infrared spectrophotometer, Bruker, Germany, in the wavelength region between 4000cm^{-1} to 400cm^{-1}. The spectra obtained for Aripiprazole, Surfactants (Poloxamer188 & Tween 80) and GMO were compared.

Standard calibration curve of Aripiprazole: 100 mg of Aripiprazole was dissolved in 100 ml of methanol. From this 10ml of was taken and was made up to 100ml with methanol. From this 10ml was taken and was made upto 100 ml with pH 7.4 phosphate buffer. From this stock solution (10µg/ml) series of concentrations 2, 4, 6 and 8µg/ml were prepared and the samples were scanned using UV-spectrophotometer. The λ_{max} was found to be at 254nm. The absorbance was noted 254nm using UV

spectrophotometer. Then a graph (Figure 1) was plotted by taking concentration on X-axis and absorbance on Y-axis which gives a straight line.

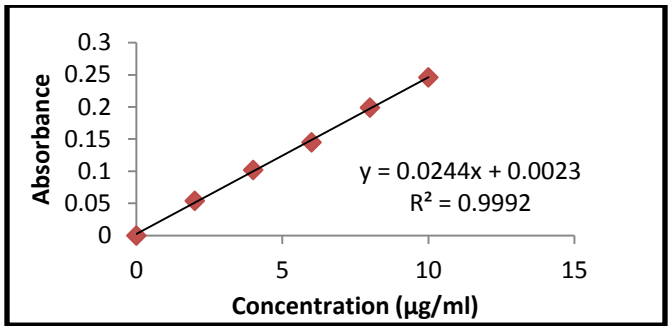


Figure 1: Standard calibration curve graph of Aripiprazole

Formulation of aripiprazole solid lipid nanoparticles: Aripiprazole loaded Solid lipid nanoparticles were prepared by high-shear homogenization technique. The lipid drug mixture was heated and maintained at 60°C, which exceeds the lipid melting temperature. An aqueous phase was prepared by dissolving Poloxamer 188 or Tween 80 (2.5% or 5% or 7.5% *w*/*v*) in double distilled water and heated to the same temperature of oil phase. Hot aqueous phase was added to lipid phase and homogenization was carried out using IKA homogenizer at 15,000 rpm for 5min. Coarse hot oil in water emulsion obtained was vibra cell ultrasonicated using probe sonicator (OrchidScientifics, Nasik) for 10 min. Aripiprazole loaded SLN dispersions were finally obtained by allowing hot nanoemulsion to cool to room temperature.

Table 1: Composition of Aripiprazole Solid lipid nanoparticles

Formulation	Aripiprazole(mg)	Glycerylmono oleate(%)	Polaxamer-188 (%)	Tween80 (%)
F1	10	2.5	1	-
F2	10	2.5	2	-
F3	10	2.5	3	-
F4	10	2.5	-	1
F5	10	2.5	-	2
F6	10	2.5	-	3
F7	10	5	1	-
F8	10	5	2	-
F9	10	5	3	-
F10	10	5	-	1
F11	10	5	-	2
F12	10	5	-	3
F13	10	7.5	1	-
F14	10	7.5	2	-
F15	10	7.5	3	-
F16	10	7.5	-	1
F17	10	7.5	-	2
F18	10	7.5	-	3

EVALUATION

Determination of average particle size and size distribution: The average particle size and size distribution of the Aripiprazole Solid lipid nanoparticle formulation was estimated using Horiba Nanopartica SZ-100. The number of particles present in the size range was considered and the average particle size was determined.

Determination of unentrapped drug (Murphy, 2006): 10 ml of SLN suspension was placed in two centrifugal tubes separately and centrifuged at 15,000 rpm at 4^0c temperature using Remi cooling centrifuge for 1hr. The clear supernatant was decanted ant the resultant precipitate was added with 5ml 7.4 phosphate buffer for 30 minutes under similar conditions. The suspension was decanted and the process was repeated again by adding 5ml phosphate buffer to ensure complete removal of unentrapped drug. The amount of the drug un entrapped was estimated at 254nm.

$$Entrapment\ efficiency = \frac{Weight\ of\ initial\ drug - Weight\ of\ free\ drug}{Weight\ of\ initial}$$

SEM and Zeta potential analysis: One drop of diluted Aripiprazole SLN suspension was placed on a stub covered with a clean glass and subjected to SEM analysis using HITACHI S-3700 N. The zeta potential of the Aripiprazole formulation was estimated using Horbia Nanopartica SZ-100.

***In vitro* diffusion studies (Inge, 2011)(BernackiJ, 2008):** *In-vitro* diffusion studies were performed by dialysis technique .SLN suspension equivalent to 5 mg of Aripiprazole was placed in dialysis bag (12,000Da– pore size) which was previously soaked overnight in distilled water and sealed at both the ends. The dialysis bag was immersed in beaker containing 250 ml of PH 7.4 phosphate buffer, maintaining at 37± 5^0c with speed of 80rpm. 5ml of samples were withdrawn at regular intervals and replaced with the fresh buffer. The amount of the drug diffused was estimated from the samples at 254 nm using UV spectrophotometer and subjected to kinetic modelling.

RESULTS AND DISCUSSION

Aripiprazole is an atypical antipsychotic drug used for the treatment of schizophrenia and bipolar disorders. The entry of this drug into the brain is constrained due to the poor permeability caused by the efflux of p-glycoproteins. The present investigation aimed to prepare Aripiprazole Solid lipid nanoparticles targeted to brain which will enhance the retention time of the drug in the brain surpassing the BBB easily. Aripiprazole Solid lipid nanoparticles were formulated by employing high speed homogenization technique using GMO and non-ionic surfactants such as Poloxomer 188 and Tween 80 as key excipients.

Compatibility studies: The compatibility studies were conducted by using IR spectral studies. The results obtained for the IR spectra of Aripiprazole, GMO and surfactants individually and the physical mixture of Aripiprazole, GMO and surfactants suggested that the characteristic peaks observed in Aripiprazole pure samples were mostly identical with the peaks in the physical mixture of Aripiprazole, GMO and surfactants adhering within their ranges without changes in the functionalities indicating its compatibility.

Figure 2: Fourier transformed Infrared spectra of Aripiprazole

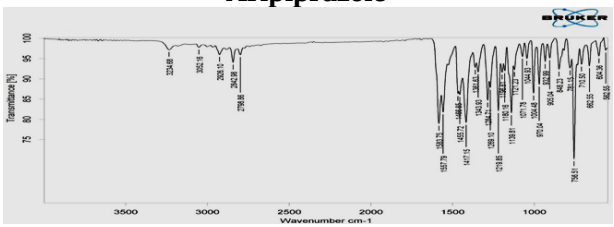


Figure 2a: IR Spectra Of Physical Mixture Of Aripiprazole + Glyceryl Mon ooleate + Poloxamer 188

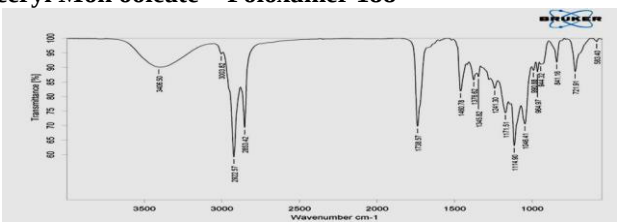
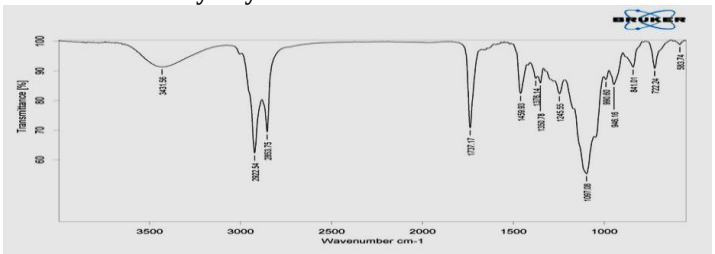


Figure 2b: IR Spectra of Physical Mixture Of Aripiprazole + Glyceryl Monooleate + Tween 80



Entrapment efficiency: Percentage swelling index of all the formulations were given in Table 2 and graphically shown in Figure 4. Aripiprazole entrapment efficiency was studied. It was observed that varying the type and concentration of either the surfactant or the lipid material used had a noticeable influence of the entrapment efficiencies of prepared SLNs. It was observed that the drug entrapment efficiency of the all formulated SLNs increased with increase in lipid up to certain concentrations (5%). This

may be due to high lipid concentrations enhances solubility of drug and so loading into Solid lipid nanoparticles. Further, the decrease in entrapment efficiency suggests the crystallization of lipid phase which produces a partial expulsion of the drug on the particle surface. Furthermore, the high viscosity at the interface produced a higher lipid concentration will cause a decrease of solvent diffusion and hence fewer lipid molecules will be carried into the aqueous phase. Therefore the formation and stabilization of lipid aggregates at these higher concentrations are reduced. The SLNs formulated using Poloxamer 188 as surfactant showed higher entrapment efficiency compared to the formulations employing Tween 80 as surfactant. This may be due to the high HLB value of Poloxamer 188 than Tween 80. The higher HLB values may enhance the encapsulation efficiency depending on the reduction of interfacial tension and enhancement of solubilization of drug. Further, when the amount of surfactant increased up to 3% in both these cases i.e, Poloxamer 188 and Tween 80, the entrapment decreased. This could be attributed due to the increase in the solubility of Aripiprazole in the aqueous phase as the percentage of surfactant increased due to the solubilization effect of emulsifier. Based on the results, the formulations F8 and F11 were found to have better entrapment efficiencies i.e. 77.73% and 70.60% respectively

Table 2: Percentage Entrapment Efficiency Of Aripiprazole Solid Lipid Nanoparticles

Formulations	Amount of drug entrapped(mg)	% Entrapment efficiency	Formulation code	Amount of drug entrapped(mg)	% Entrapment efficiency
F1	82.20	55.00	F10	98.37	65.58
F2	92.89	62.4	F11	112.00	70.60
F3	80.05	53.33	F12	90.65	60.43
F4	72.70	48.50	F13	92.68	61.78
F5	84.50	56.15	F14	104.80	72.92
F6	76.30	51.08	F15	80.48	53.65
F7	106.91	71.27	F16	88.21	58.81
F8	116.60	77.73	F17	102.03	68.02
F9	95.12	63.41	F18	82.11	54.80

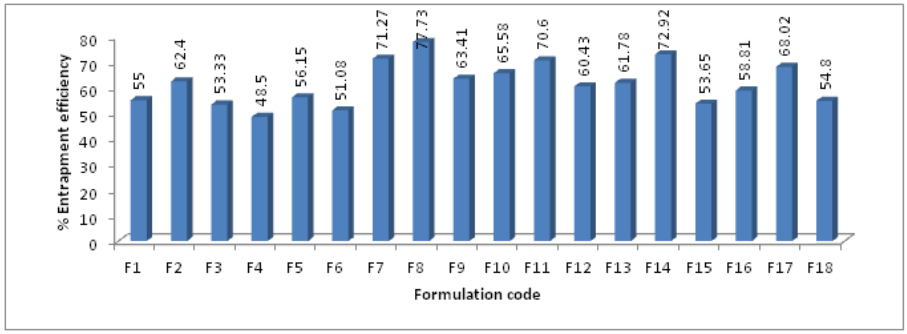


Figure 3: Plot of Percentage Entrapment Efficiency of Aripiprazole SLNs

***In-vitro* diffusion studies:** The dialysis method was used to investigate the in vitro Aripiprazole release from solid lipid nanoparticle formulations. All the formulations were subjected to *in-vitro* diffusion studies in pH 7.4 phosphate buffer. The release profiles till the end of 72 hrs are shown in Figure 5a-f and given in Table 3. Based on the release profile the formulations F8 & F11 showed highest release i.e. 81.34% and 85.86% respectively at the end of 72 hrs. The dissolution data were fitted to popular release kinetic equations. Analysis of the drug release data as per zero order and first order kinetic models indicating that all the formulations followed first order kinetics and different in-vitro dissolution parameters such as dissolution rate constant (K), time required for 50% drug dissolution (T50) and 90% drug dissolution (T90) were determined and presented in Table 4. Based on the release exponent 'n' values, it was observed that the formulation mechanism release pattern was governed by predominant non-fickian diffusion.

Table 3: *In-vitro* drug diffusion data

Table 3.1: *In vitro* drug diffusion data of Aripiprazole Solid lipid nanoparticles with 2.5% GMO and Poloxamer 188 (F1, F2, F3), 2.5% GMO and Tween80 (F4, F5, F6)

Time	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
1	3.70±0.34	4.08±0.18	3.25±0.42	4.80±0.16	2.95±0.44	6.95±0.28
2	5.69±0.51	7.86±0.27	4.27±0.56	6.74±0.38	4.14±0.12	8.94±0.44
4	9.66±0.21	11.91±0.54	9.20±0.12	10.75±0.16	9.51±0.48	12.82±0.36
8	19.28±0.34	19.38±0.65	17.71±0.27	20.45±0.21	17.60±0.18	22.69±0.44
12	29.01±0.56	27.38±0.16	33.36±0.12	30.25±0.42	29.38±0.27	33.14±0.36
24	38.86±0.54	37.71±0.25	42.18±0.18	40.724±0.21	39.57±0.14	48.60±0.28
48	53.64±0.18	55.34±0.46	55.69±0.26	55.04±0.16	48.91±0.32	59.76±0.14
72	66.13±0.21	74.13±0.14	70.81±0.52	73.01±0.36	64.10±0.24	68.55±0.21

Table 3.2: *In vitro* drug diffusion data of Aripiprazole Solid lipid nanoparticles with 5% GMO and Poloxamer 188 (F7, F8, F9), 5% GMO and Tween80 (F10, F11, F12)

Time	F7	F8	F9	F10	F11	F12
0	0.00	0.00	0.00	0.00	0.00	0.00
1	5.62±0.18	7.26±0.36	3.26±0.14	6.24±0.18	5.62±0.21	5.21±0.16
2	6.45±0.34	9.56±0.5	6.51±0.26	9.23±0.34	9.84±0.16	7.98±0.38
4	9.61±0.27	15.42±0.46	10.08±0.32	14.17±0.21	13.14±0.18	12.23±0.42
8	16.34±0.46	26.32±0.18	18.47±0.16	22.87±0.18	23.46±0.16	21.40±0.36
12	25.28±0.14	41.98±0.26	32.41±0.14	44.15±0.42	32.64±0.44	31.06±0.44
24	49.15±0.34	55.17±0.18	41.44±0.27	53.17±0.18	42.66±0.36	40.25±0.14
48	64.75±0.16	69.83±0.44	58.63±0.18	65.44±0.21	62.02±0.18	54.02±0.32
72	75.21±0.21	81.34±0.16	67.11±0.44	78.76±0.36	85.86±0.14	72.24±0.28

Table 3.3: *In vitro* drug diffusion data of Aripiprazole Solid lipid nanoparticles with 7.5% GMO and Poloxamer 188 (F13, F14, F15), 7.5% GMO and Tween80 (F16, F17, F18)

Time	F13	F14	F15	F16	F17	F18
0	0.00	0.00	0.00	0.00	0.00	0.00
1	3.05±0.34	2.95±0.32	2.13±0.44	3.77±0.14	3.81±0.36	2.95±0.24
2	4.96±0.16	4.14±0.24	5.25±0.26	6.31±0.16	5.72±0.27	4.86±0.18
4	9.31±0.12	7.51±0.21	10.24±0.18	10.73±0.21	9.78±0.18	8.79±0.14
8	15.54±0.18	17.60±0.12	19.91±0.27	15.35±0.14	19.38±0.44	18.34±0.21
12	22.45±0.16	28.25±0.21	27.68±0.36	23.44±0.26	33.31±0.30	27.99±0.21
24	27.86±0.27	38.52±0.32	34.43±0.18	30.12±0.14	41.86±0.42	37.53±0.32
48	46.03±0.14	52.55±0.16	48.30±0.21	44.11±0.18	49.64±0.14	52.49±0.27
72	58.49±0.14	63.05±0.28	60.38±0.32	56.43±0.26	65.25±0.32	61.37±0.18

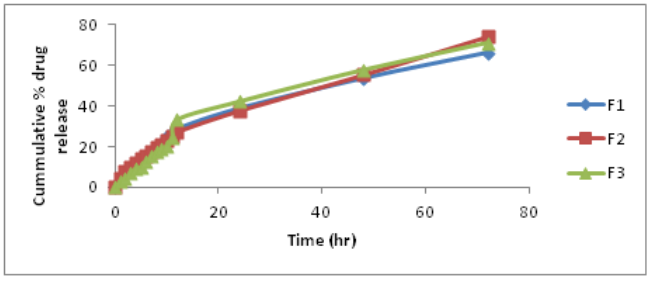


Figure 4a: *In vitro* diffusion profile of Aripiprazole Solid lipid nanoparticles with 2.5% GMO and Poloxamer 188

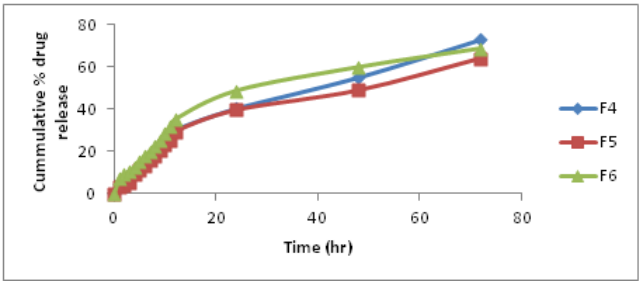


Figure 4b: *In vitro* drug diffusion profile of Aripiprazole Solid lipid nanoparticles with 2.5% GMO and Tween80

Table 4: *In vitro* drug diffusion kinetics of Aripiprazole Solid lipid nanoparticles

Formulation code	Correlation coefficient (r^2)				Release kinetics			Exponential coefficient (n)
	Zero order	First order	Huguchi	Peppas	K (Hr^{-1})	$T_{50\%}$ (Hr)	$T_{90\%}$ (Hr)	
F1	0.8366	0.9575	0.9820	0.9845	0.0166	41.7	138.7	0.7067
F2	0.8817	0.9831	0.9849	0.9954	0.0189	36.6	121.5	0.6568
F3	0.9311	0.9917	0.9614	0.9940	0.0177	37.8	123.9	0.6418
F4	0.8482	0.9720	0.9841	0.9906	0.0188	36.8	122.2	0.6690
F5	0.8621	0.9594	0.9684	0.9738	0.0161	43.2	143.4	0.8038
F6	0.7231	0.9179	0.9832	0.9836	0.0190	36.6	121.5	0.5980
F7	0.9181	0.9882	0.9561	0.9842	0.0209	33.2	110.3	0.6971
F8	0.7478	0.9655	0.9834	0.9846	0.0262	26.4	87.9	0.6226
F9	0.8318	0.9543	0.9761	0.9823	0.0177	39.2	130.2	0.7262
F10	0.7761	0.9580	0.9764	0.9836	0.0235	29.5	97.9	0.6330
F1I	0.8611	0.9806	0.9857	0.9931	0.0256	27.1	89.9	0.6312
F12	0.8106	0.9596	0.9879	0.9888	0.0186	37.3	123.8	0.6319
F13	0.8786	0.9649	0.9826	0.9908	0.0132	52.6	174.6	0.6896
F14	0.8500	0.9511	0.9706	0.9721	0.0152	45.5	151.1	0.7921
F15	0.7829	0.9233	0.9646	0.9842	0.0145	47.8	158.8	0.7448
F16	0.8406	0.9467	0.9913	0.9941	0.0127	54.6	181.3	0.6193
F17	0.8621	0.9594	0.9684	0.973	0.0161	43.2	143.4	0.8038
F18	0.8301	0.9440	0.9785	0.9806	0.0152	45.6	151.5	0.7462

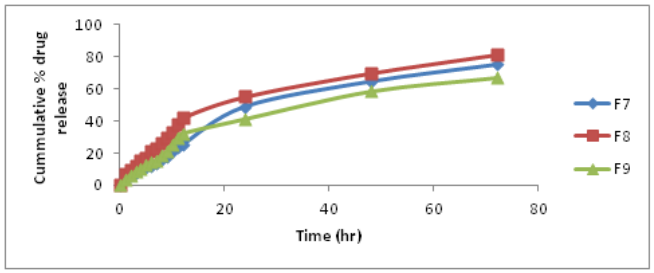


Figure 4c: *In vitro* drug diffusion profile of Aripiprazole Solid lipid nanoparticles with 5% GMO and Poloxamer 188

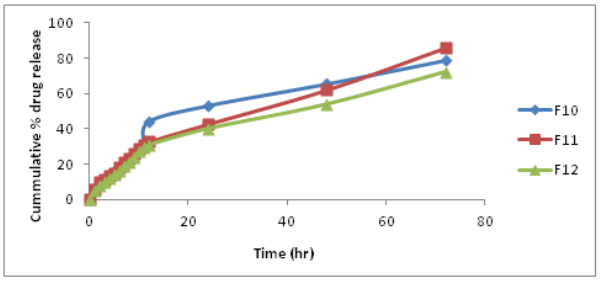


Figure 4d: *In vitro* drug diffusion profile of Aripiprazole Solid lipid nanoparticles with 5% GMO and Tween80

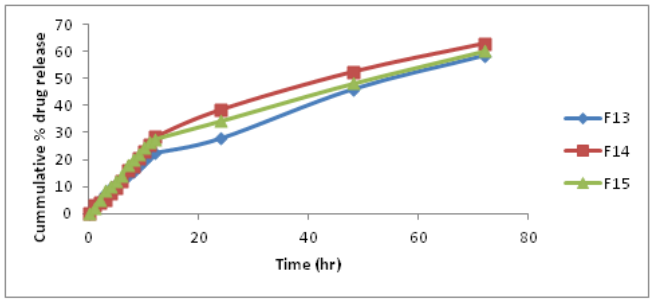


Figure 4e: *In vitro* drug diffusion profile of Aripiprazole Solid lipid nanoparticles with 7.5% GMO and Poloxamer 188

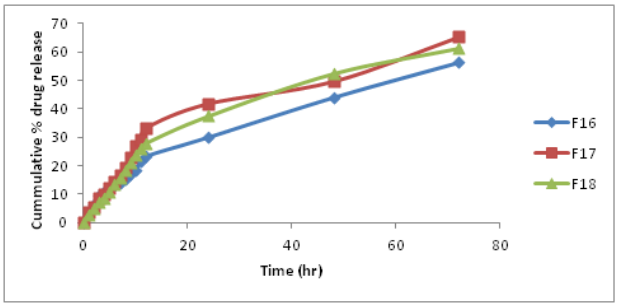


Figure 4f: *In vitro* drug diffusion profile of Aripiprazole Solid lipid nanoparticles with 7.5% GMO and Tween80

Particle size analysis: The particle size distribution of Aripiprazole Solid lipid nanoparticle formulation was estimated using Horiba Nanopartica SZ-100 particle size analyser range. The Particle size range of Solid lipid nanoparticles formulations F8 & F11 were ranged between 120nm to 400 nm and 150nm to 520nm with the average particle size around 202.6nm and 263.9nm respectively indicating well within the nanoparticle limits.

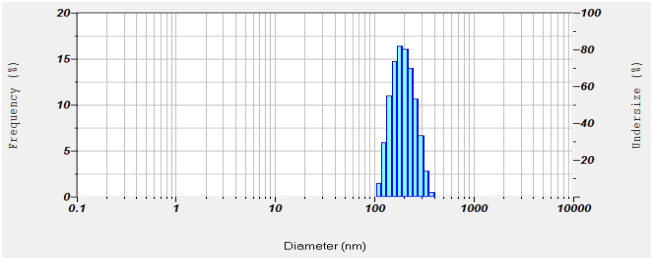


Figure 5a: Size distribution of optimized formulation (F8)

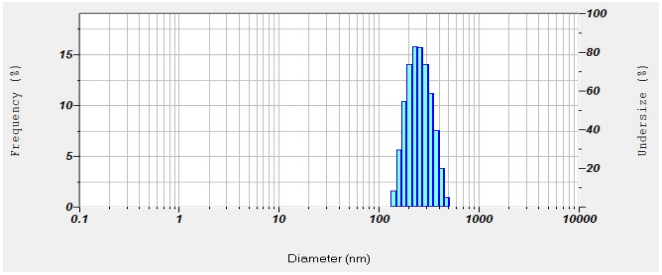


Figure 5b: Size distribution of optimized formulation (F11)

SEM and Zeta potential analysis: SEM analysis reports revealed that the nanoparticles formed were spherical in shape was shown in Figure 6a-b. The zeta potential is an indication of the stability and charge present on the surface of the Solid lipid nanoparicles. The zeta potential values of the optimized formulations F8 and F11 were found to be -28.9 mV and -33.1mV was shown in Figure 7a-b. This value indicates the high negative surface charge on niosomes which leads to high stability because of the anticipated surface repulsion between similar charged particles hence inhibiting aggregation of the nanoparticles.

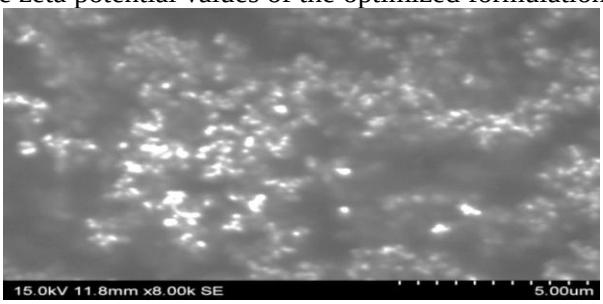


Figure.6a.SEM photograph of optimized formulation (F8)

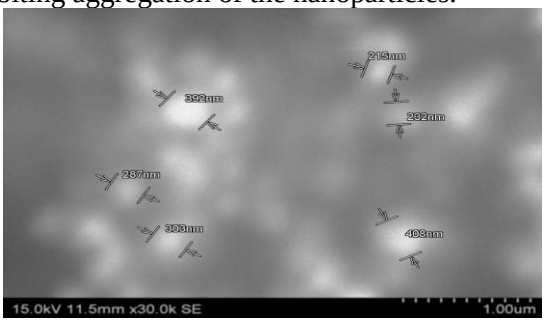


Figure.6b.SEM photograph of optimized formulation (F8) (high magnification)

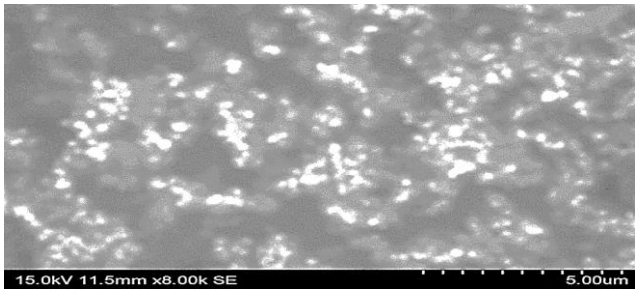


Figure 6c: SEM photograph of optimized formulation (F11)

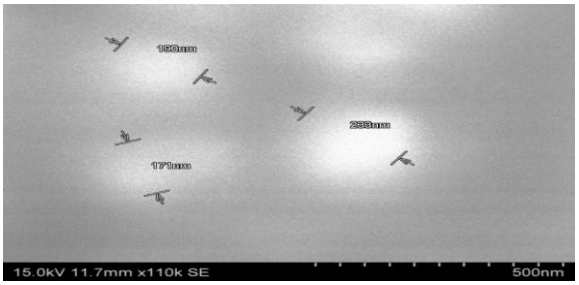


Figure 6d: SEM photograph of optimized formulation (F11) (high magnification)

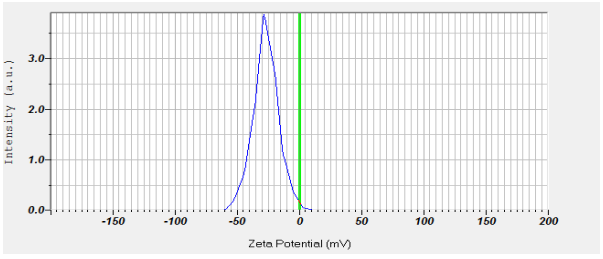


Figure 7a: Zeta potential of optimized formulation (F8)

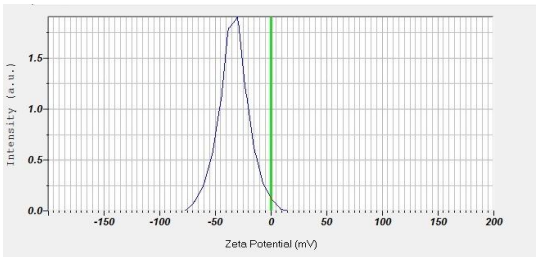


Figure 7b: Zeta Potential of optimized formulation (F11)

CONCLUSION

Aripiprazole Solid lipid nanoparticles were formulated with a view to target the drug to the brain surpassing the BBB and increase the retention time in brain for better therapy of CNS disorders. Aripiprazole Solid lipid nanoparticles were formulated with different non-ionic surfactants and GMO with varying concentrations. The formulations F8 & F11 with 2% surfactants i.e. Poloxamer 188 and Tween 80 respectively showed high entrapment efficiency and better drug delivery.

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