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Research Article

Formulation and Evaluation of Bromocriptine Mesylate Loaded Solid Lipid Nanoparticles for the Treatment of Parkinson's Disease

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ABSTRACT

The aim of this study was to formulate, characterize and evaluation of solid lipid nanoparticles (SLNs) of practically insoluble bromocriptine mesylate to enhance its solubility and to improve its oral bioavailability by avoiding first pass effect as well as to produce control release action of the drug from the SLNs for an efficient management of Parkinson's Disease in addition to an improvement of the patient compliance. The SLNs were prepared by the Emulsification-Solvent Evaporation method using glycerylbehenate as lipid and pluronic F-68 were used as stabilizer and Ethanol and chloroform used as a solvent. The prepared SLNs then tested for entrapment efficiency, solubility analysis, *in vitro* drug release then characterize (Scanning Electron Microscope (SEM), particle size distribution, zeta potential) and evaluate their flow property, uniformity of weight, *in vitro* disintegration test, drug content, *in vitro* drug release, release kinetics, *in vivo* studies and stability studies of optimized SLNs and filled in Hard Gelatin Capsules (HGC). The SLNs release the drug in contolled manner upto 12hrs. From the animal study, it was concluded that the group treated with optimized SLN.G5 showed better and sustained reduction in parkinson's symptoms as compared to control group and the group treated with pure drug formulation, indicated improvement in bioavailability of drug.

INTRODUCTION

Parkinson's Disease is recognized as the second most common, progressive neurodegenerative disorder in the world after Alzheimer's and it manifested clinically by motor symptoms, including rigidity, resting tremor and bradykinesia in the limbs followed by postural instability. The treatment of PD is restricted by an insufficiency in delivering therapeutic drugs into the brain relating to highly limited transport of compounds across the blood–brain barrier. A low molecular mass 400-500 Da and high lipophilic drugs can only cross the blood brain barrier.

To overcome this obstruction, a variety of colloidal carriers have been implemented like liposomes, polymeric nanoparticles, solid lipid nanoparticles and dendrimers. Among these solid lipid nanoparticles become an important area of research in the field of drug delivery into the brain in a sustained period of time [1].

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SLNs are sub-micron colloidal carrier of 50-1000nm size range, which are composed of physiological lipid dispersed in water or in an aqueous surfactant solution. They are made of solid hydrophobic core having a monolayer of phospholipid coating. The solid core contains the drug dissolved or dispersed in the solid high melting fat matrix. The hydrophobic chains of phospholipids are embedded in the fat matrix. They have potential to carry lipophilic or hydrophilic drugs or diagnostics [2].

Bromocriptine (BRC) an ergot derivative with dopamine receptor agonist activity has been widely used clinically to delay and minimize the deleterious motor fluctuations associated with long term levodopa treatment in PD. In addition to its action as a DA receptor agonist, BRC is known to directly and indirectly protect dopaminergic cells. BRC has also been reported to have antioxidant effects, inhibit free radical formation and scavenge free radicals, and retard apoptosis, which is probably accelerated in PD [3].

Bromocriptine is rapidly absorbed, after oral administration, the bioavailability of the drug is

28% of the oral dose consumed, and the plasma protein binding amounts to 96%. It is highly distributed in the liver, stomach, and intestine. The drug is extensively metabolized in the liver. The fate of bromocriptine mesylate primarily involves with renal excretion of two major metabolites accounting for 6% of the total dose [4].

The aim of this study was to formulate and evaluate solid lipid nanoparticles of practically insoluble bromocriptine to enhance its solubility of drug and to improve its oral bioavailability of drugs by avoiding first pass effect as well as to produce control release action of drug from the lipid matrix for an efficient management of Parkinson's disease in addition to an improvement of the patient compliance to this patient-friendly dosage form.

MATERIALS AND METHODS

Material

Bromocriptine mesylate (BCM) was procured gift sample from Zyduscadila, Ahmedabad. Glycerylbehenate and Pluronic F-68 procured from Saimirrainnopharmpvt.ltd, Chennai. All other chemicals and solvents were of analytical grade.

Method

Determination of Saturation Solubility

The solubility of Bromocriptine Mesylate was determined by dissolving the excess amount of drug in different medium 0.1N HCl, distilled water at room temperature and kept for 48h with regular shaking. The solutions were centrifuged then filtered and examined by using UV-spectrophotometer at its λ max, and from this absorbance, the concentration of that saturated solution was determined which represents the solubility of bromocriptine mesylate in these solution [5].

Partition Co-efficient Studies

The partitioning behavior of BCM was determined in lipid like Glyceryl Behenate. 10 mg of Bromocriptine was added in a mixture of melted lipid (1 g) and 1 ml of hot 0.1 N HCl and shaken for 30 minutes in a magnetic stirrer using a hot water bath maintained 10°C above the melting point of the lipid. The aqueous phase of the above mixture was separated from the lipid by centrifugation at 10000 rpm for 30 minutes in a high-speed cooling centrifuge. The clear supernatant was suitably diluted with 0.1N HCl and the Bromocriptine content was quantified using UV-visible spectrophotometer at 305 nm against a blank. The partition coefficient of Bromocriptine in lipid/ 0.1 N HCl was calculated using Equation [6]:

$$\text{Partition coefficient} = C_{BCM}/C_A$$

C_{BCM} is the amount of Bromocriptine in lipid and C_A is the amount of Bromocriptine in 0.1 N HCl.

Formulation of Solid Lipid Nanoparticles [7]

Bromocriptine loaded solid lipid nanoparticles is prepared by Emulsification/Solvent Evaporation (ESE) Method. Drug and lipids of different ratio are weighed accurately for the preparation of solid lipid nanoparticles. In this technique the Lipophilic material and hydrophobic drug were dissolved in a water immiscible organic solvent. The aqueous medium is prepared by dissolving Pluronic F-68 in distilled water and kept for stirring in magnetic stirrer for 15 mins heated above its melting temperature of lipid. Upon evaporation of the solvent, lipid phase is slowly added into aqueous phase under continuous stirring. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase the mixture homogenization was performed 2500 rpm by using high speed homogenizer for 60 min. The coarse oil in water emulsion was the obtained to ultrasonicated using probe sonicator the amplitude in a pulse regime (2s on, 1s off) during 10 min. Forming nanoemulsion was centrifuged in high speed cooling centrifuge (10,000 rpm) at 4°C. The nanoparticles are separated and subjected to lyophilization [7].

Table 1: Formulation table of Bromocriptine solid lipid nanoparticles

Formulation Code	Drug: Lipid Ratio (BCM:Glycerylbehenate)	Pluronic F-68 (%W/V)
SLN.G1	1:1	2
SLN.G2	1:3	2
SLN.G3	1:5	2
SLN.G4	1:7	2
SLN.G5	1:9	2
SLN.G6	1:12	2

Entrapment Efficiency

The entrapment efficiency of solid lipid nanoparticles dispersion was determined by the centrifuge method. Bromocriptine loaded SLNs dispersion was centrifuged at 20000 rpm for 30 min using high speed cooling centrifuge at 4°C.The clear supernatant was removed from the residue and after suitable dilution the

absorbance was recorded at 305 nm using UV Spectrophotometer.

The percentage entrapment efficiency (%EE) is calculated by following formula [7].

$$\%EE = \frac{\text{Total amount of drug taken} - \text{Amount of drug in supernatant}}{\text{Total amount of drug taken}} \times 100$$

Solubility Studies of SLNs Formulations

The solubility of the SLNs Formulation was determined in various medium (0.1 N HCl and Distilled water).

***In Vitro* Drug Release Studies**

In vitro release rate studies of Bromocriptine Mesylate loaded SLNs formulations were carried out by using dissolution test apparatus USP Type-I (basket). BCM loaded SLNs filled equivalent amount in capsule and placed in a dissolution jar. 0.1 N HCl (500 ml) was used as dissolution medium and rotated at 120 rpm.10 ml of samples were withdrawn predetermined intervals of 1hrs up to 12hrs and replaced with equal amount of 0.1 N HCl for further dissolution testing the absorbance determined by spectrophotometrically at 305 nm [8].

Surface Morphology of SLNs

Scanning electron microscopy (SEM) is an excellent tool for physical observation of morphological features of particle both initially and degradation process. It is helpful to examine particle shape and surface characteristics such as surface area and bulk density. The sample was mounted directly onto the SEM sample holder using double sided sticking tape and images were recorded at different magnifications at acceleration voltage of 10 kV using scanning electron microscope.

Particle size and Polydispersity

Particle size (z- average diameter), polydispersity index (as a measure of the width of the particle size distribution) of Bromocriptine mesylate loaded SLN dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy(PCS) using a Malvern Zetasizer 3000 Nano S (Malvern instruments, UK) at 25°C. Prior to measurements all samples were diluted using ultra – purified water to yield a suitable scattering intensity. The diluted nanoparticle dispersion was poured into the disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement.

Zeta Potential

Zeta Potential is a crucial factor to evaluate the stability of colloidal dispersion. Surface charge on the Bromocriptine mesylate loaded SLNs were determined using Malvern Zetasizer. 1 ml of sample of Bromocriptine mesylate suspension was filled in clear disposable zeta cell, ensured there was no air bubble within the sample, and the system was set at 25°C temperature and the test can be carried out [9].

Preformulation Study of Optimized SLNs

Flow Property Measurements

The flow properties are critical for an efficient capsule filling operation. A good flow of the powder or granules is necessary to assure efficient mixing and acceptable weight uniformity for the capsules. The flow property measurements include bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio. The flow property measurements of Bromocriptine SLNs are determined.

Optimized SLNS Filled in Hard Gelatin Capsules

The optimized Bromocriptine solid lipid nanoparticles unstable in suspension form so it is lyophilized and converted into powder form. The dried powder is filled into "2" size hard gelatin capsules and each capsules containing 5 mg equivalent of Bromocriptine.

Evaluation of Optimized Capsules

Uniformity of Weight

Intact capsule were weighed. The capsules were opened without losing any part of the shell and contents were removed as completely as possible. The shell was washed with ether and the shell allowed to stand until the odour of the solvent was no longer detectable. The empty shell was weighed. The average weight was determined. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation and none deviates by more than twice that percentage.

Drug Content

Five capsules were selected randomly and the average weight was calculated. The powder is removed completely. An amount of powder was equivalent to 5 mg made upto 100 ml with 0.1 N HCl. It was kept for overnight. 1 ml of solution

was diluted to 100 ml using 0.1 N HCl in separate standard flask. The absorbance of solution was recorded at 305 nm [10].

In Vitro Drug Release Studies

In vitro release rate studies of Bromocriptine Mesylate loaded SLNs formulations were carried out by using dissolution test apparatus USP Type-I (basket). BCM loaded SLNs filled equivalent amount in capsule and placed in a dissolution jar. 0.1 N HCl (500 ml) was used as dissolution medium and rotated at 120 rpm.10 ml of samples were withdrawn predetermined intervals of 1hrs up to 12hrs and replaced with equal amount of 0.1 N HCl for further dissolution testing the absorbance determined by spectrophotometrically at 305 nm [8].

Kinetics of Drug Release

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of drug release rate kinetics of the dosage form, the obtained data were fitted into Zero Order, First Order, Higuchi, Hixon- Crowell release model and Korsmeyer-Peppas model [11].

In-Vivo Study

The antiparkinson's studies was performed using healthy swiss albino mice (20-25 gm) obtained from KMCP Madurai and were housed in polypropylene cages, with laboratory diet and water. Animals were acclimatized for period of 24 h to ensure their suitability for study. Approval number: IAEC/KMCP/62/2019.

Pharmacodynamics Study

Animals were randomly distributed into four different groups having four animals in each group. Group-1 animals served as control and were administered orally with normal saline as vehicle. Group-2 animals were administered with haloperidol at a dose of 2 mg/kg. Group-3 animals were given a combined treatment of haloperidol and Bromocryptine mesylate solution at a dose of 0.22 mg/kg, (intra peritoneal). A lag time of 15 min was maintained between Haloperidol and Bromocryptine mesylate solution. Group-4 animals were given a combined treatment of haloperidol 2 mg/kg(intra peritoneal) and solid lipid nanoparticles formulation G5 dissolved with DMSO at a dose of 10 mg/kg (intra peritoneal) after 15 min. Behavioral quantification was done before the sacrifice of animals.

Catalepsy

The bar test was used for measuring catalepsy. In the bar test, animal was placed on the apparatus such that its forepaws rested on the bar and their hind limbs were on the platform. After the animal was positioned properly the experimenter released its hold. Catalepsy was measured by the time the animal maintained its position on the bar. When the animals removed one paw from the bar the stopwatch was stopped and the time noted. Animals were observed for a maximum cutoff time for bar test which was fixed for 5 min. Following assessment the animals were returned to their home cages [12].

Akinesia

Akinesia was measured by noting the akinesia (difficulty in initiating movement) in seconds (s) to move all the four limbs and the test was terminated if the akinesia exceeded 120 s. Each animal was initially acclimatized for 5 min on a wooden elevated (30 cm) platform (40 ×40 cm) used for measuring akinesia in mice. Using a stopwatch, the time taken by the animal to move all the four limbs was recorded [13].

Biochemical Estimation

GSH Estimation Assay

Glutathione (GSH) was estimated as the total non-protein sulphydryl groups. In this method the 5, 50-dithiobis-(2-nitro-benzoic acid) (DTNB) is reduced by the –SH groups of GSH to form one mole of 2-nitro-5-mercaptobenzoic acid per mole of –SH. The nitromercaptobenzoic acid anion released has an intense yellow color, which can be used to measure –SH groups at 412 nm. A quantity of 2 ml of 10% of the homogenate was mixed with 2.5 ml of 0.02 M ethylene diamine tetra acetic acid solution and 2 ml of the resultant mixture was further mixed with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid (TCA) solution. Following centrifugation at 3000 rpm for 15 min, 2 ml of the supernatant was mixed with 4 ml of Tris buffer (0.4 M, pH 8.9) and 0.1 ml of DTNB solution and shaken. The absorbance of the yellow color complex was read at 412 nm. GSH was used as a standard to calculate the content of GSH which is expressed as micro- moles of GSH per milligram of protein [14].

TBARSs Estimation

Measurement of lipid peroxidation by determination of brain malondialdehyde (MDA) content was performed by a thiobarbituric acid method. One ml of suspension was taken from

the 10% brain homogenate and was diluted to 4 ml with 0.15 M potassium chloride solution. This was followed by the addition of 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA (thiobarbituric acid) reagent. The tubes were then covered with aluminium foil and kept in shaking water bath for 30 min at 80°C. After 30 min tubes were taken out and kept in ice cold water for 30 min. After cooling, the contents were centrifuged at 3000 rpm for 15 min. The amount of TBARS was assessed by measuring the absorbance of the supernatant at 540 nm using a spectrophotometer against a blank with no homogenate. The results were expressed as nanomoles of MDA per milligram of protein [15, 16].

Statistical Analysis

Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad prism software). All results are expressed as mean ± SEM. Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett's t-test. The values were considered statistically significant, when $p < 0.05$ and differences of $p < 0.01$ were considered highly significant.

Stability Studies

The optimized Bromocriptine solid lipid nanoparticles filled in hard gelatin capsules and the optimized formulations were studied for stability for 3 months. The optimized formulations were divided into 3 groups. One group was kept at refrigeration (4±1°C) temperature, second group at room temperature and the third group at 40±2°C, 70±5% RH temperature. Every month, the formulations were withdrawn and analyzed for change in physical appearance, drug content, *in-vitro* release kinetics and entrapment efficiency. The purpose of stability testing was to provide evidence on how the quality of a drug substance or nanoparticles varies with time under influence of varies environmental factors such as temperature, humidity and light [17].

RESULTS AND DISCUSSION

Saturation Solubility of Bromocriptine Mesylate

The solubility of the drug at acidic pH was significantly higher than in that of distilled water, as shown in the Table 2, this can reflect the alkaline nature of the drug.

Table 2: Saturation solubility of BCM in 0.1N HCl and Distilled water

Solvent	0.1 N HCl (pH 1.2)	Distilled water (pH 7)
Solubility(µg/ml) mean ±SD	23.60±0.2702	5.35±0.8815

Mean ±SD (n= 3)

The solubility of the drug at acidic pH was significantly higher than in that of distilled Water, The results can reflect the alkaline nature of the drug.

Partition Co-efficient Studies

Table 3: Partition co-efficient value of different lipids

Lipid	Partition co-efficient
GlycerylBehenate (GB)	2.53±0.3636

Mean ±SD (n= 3)

The Partition coefficient value was found to be 2.53.

Evaluation of Bromocriptine Loaded SLNs

Drug Entrapment Efficiency

The formulated Bromocriptine solid lipid nanoparticles are characterized for Entrapment efficiency and the results were tabulated below.

Table 4: Characterization of Bromocriptine loaded SLNs

Formulation Code	Entrapment Efficiency (%)
SLN.G1	79.25±0.7528
SLN.G2	81.89±0.9335
SLN.G3	86.56±0.7710
SLN.G4	88.26±0.9347
SLN.G5	90.94±0.5706
SLN.G6	89.18±0.8129

Mean ±SD (n= 3)

The entrapment efficiency of the formulations SLN.G1 to SLN.G6 was observed in between 79.25% to 90.94%. SLN.G5 formulation prepared with BCM, Glycerylbehenate (1:9) and 2% stabilizer shows highest 90.94% entrapment efficiency comparatively with other formulations. The results show that the increase in lipid concentration, increased the drug entrapment efficiency. This may be due to higher concentration of the lipid would provide more space and also reduces the escaping of drug into the external phase. The entrapment efficiency of the formulations SLN.G1 to SLN.G6 was observed in between 74.72% to 87.04%.

Solubility Studies of SLNs Formulations

Table 5: Solubility of Solid Lipid Nanoparticesin various medium

S. No	Formulation Code	Solubility Medium	
		Distilled Water pH 7(mg/ml)	0.1 N HCl pH 1.2 (mg/ml)
1	Pure drug	0.005345	0.0236
2	SLN.G1	8.4025	8.7685
3	SLN.G2	9.6088	9.1172
4	SLN.G3	12.2344	12.3718
5	SLN.G4	`16.3502	18.7600
6	SLN.G5	23.304	25.2160
7	SLN.G6	15.7115	15.1035

The solubility of Bromocriptine mesylate in distilled water and 0.1 N HCl were found to be in the range from 0.005345 to mg/ml and 0.0236 to mg/ml. The solubility of all formulation were improved (from insoluble to slightly soluble) compared to pure drug of Bromocriptine. Among all the formulations SLN.G5 show higher solubility in distilled water and 0.1 N HCl. Thus the solubility of formulation SLN.G5 in Distilled water and 0.1 N HCl were improved (884 & 1068) fold respectively.

In-Vitro Drug Release Studies

The formulated SLNs preparation containing drug and lipids (Bromocriptine and Glycerylbehenate) in the ratio of (1:1, 1:3, 1:5, 1:7, 1:9 and 1:12) were evaluated for drug release and results were tabulated below.

Among all the formulations SLN.G5 formulation shows 98.75% of drug release up to 12thh in controlled manner. Based on the entrapment efficiency and *in vitro* drug release SLN.G5 was selected as optimized formulations.

Table 6: *In vitro* drug release for all formulations

Time (h)	*In Vitro* Drug Release For Solid Lipid Nanoparticles (SLNs) Formulations					
	G1	G2	G3	G4	G5	G6
1	19.35	13.54	16.50	14.16	12.78	13.44
2	27.18	23.60	23.49	21.15	21.06	19.74
3	34.87	32.73	33.76	27.27	31.89	27.15
4	41.91	37.66	43.60	39.95	42.26	46.62
5	49.54	45.77	51.07	50.60	51.06	51.57
6	55.32	52.66	62.66	59.55	56.68	57.29
7	63.18	63.45	65.98	65.98	63.02	62.41
8	77.40	75.70	72.97	70.99	71.30	72.61
9	85.50	82.77	78.91	78.57	75.69	78.52
10	97.68	91.27	84.37	90.87	84.27	84.43
11	-	98.44	92.37	93.13	91.93	92.71
12	-	-	97.48	95.72	98.75	95.43

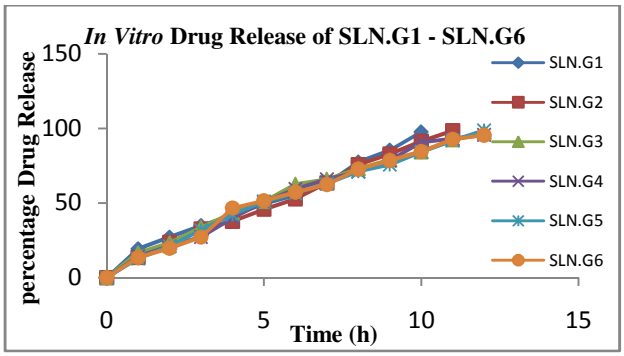

Figure 2: *In vitro* drug release study of SLNs (SLN.G1 to SLN.G6)

Scanning Electron Microscope (SEM) Analysis

The shape and surface morphology of optimized formulations SLN.G5 was observed in scanning electron microscopy. The SEM image of optimized formulations SLN.G5 shows the solid lipid nanoparticles are discrete, spherical and with smooth surface.

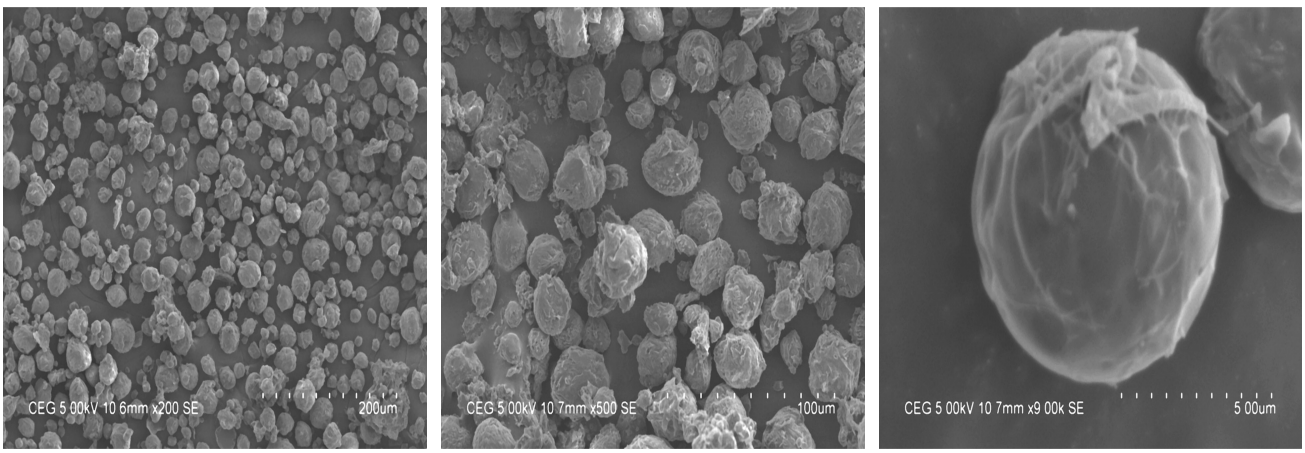

Figure 3: SEM Image of optimized formulation SLN.G5

Particle Size Characterization

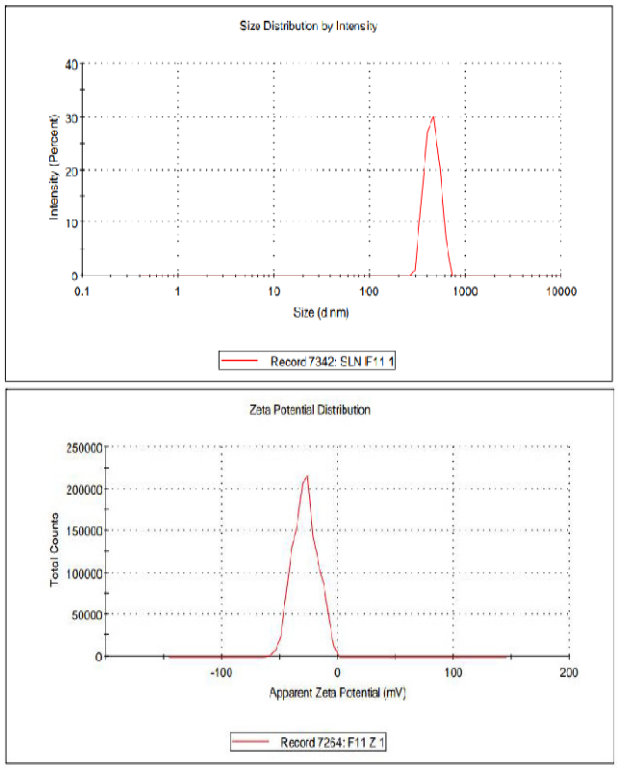

Figure 4: Particle size distribution, Zeta potential of SLN.G5

Table 7: Particle Size, Polydispersity and Zetapotential

F. Code	Particle size (nm)	Polydispersity	Zeta potential
SLN.G5	486.1 nm	0.802	-27.9 mV

Preformulation Studies of Optimized SLNs

After Lyophilization the preformulation studies of optimized Solid Lipid Nanoparticles formulations were carried out to check the flow property. The optimized formulations SLN.G5 was evaluated for flow properties and the results were shown in table.

The optimized formulations SLN.G5 shows good flow property was observed compared with Bromocriptine pure drug.

Table 8: Flow property measurements of optimized SLNs

Formulation Code	Pure Drug	SLN.G5
Bulk density (g/ml)	0.2846±0.008	0.2371±0.011
Tapped density (g/ml)	0.4028±0.01	0.2742±0.025
Carr's Index (%)	29.29±1.162	13.24±0.557
Hausner's ratio	1.4157±0.053	1.1552±0.062
Angle of repose (θ)	44.25±1.849	31.00±1.170

Mean ±SD (n= 3)

Optimized Bromocriptine SLNs Filled in HGC Capsules

The optimized SLNs were filled into "2" size hard gelatin capsules (HGC) without adding glidant or exicipients because of good flow properties can be observed. The filled each capsules containing 5 mg equivalent quantity of Bromocriptine.

Post Formulation Studies for Bromocriptine Solid Lipid Nanoparticulate Capsules(C-SLN)

Table 9: Uniformity of Weight of contents in capsules

Formulation code	Average weight of capsules (g)
C-SLN.G5	0.0117±0.0054

Mean ±SD (n= 3)

The Bromocriptinesolid lipid nanoparticulate capsules comply with the official test for Uniformity of weight.

Table 10: Drug Content of optimized Formulations

Formulation code	Drug content (%)
C-SLN.G5	98.92±.094889

Mean ±SD (n= 3)

The Drug content of the optimized formulation (SLN.G5) was observed 98.92%. The drug content was within the limits (not less than 90% and not more than 110%). It complies with standard.

In Vitro Release of Optimized Formulations and Pure Drug

The *in vitro* release data of optimized formulation SLN.G5 was compared with pure drug Bromocriptine and the results were tabulated below.

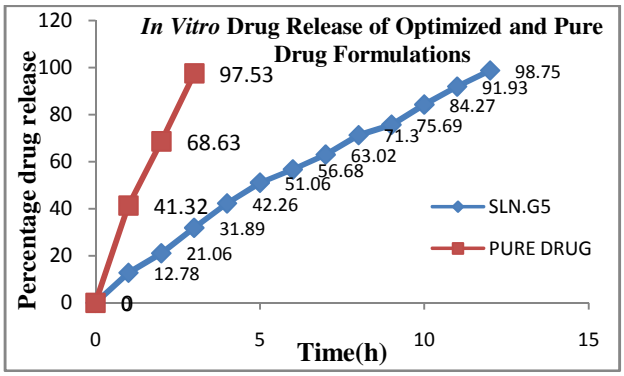

Figure 5: *In vitro* drug release of optimized formulations and pure drug.

The *in vitro* release profile of optimized BromocriptineSLN.G5 shows (98.75%) controlled release up to 12thh, but pure drug shows (97.53%) up to 3rd h.

Release Kinetics of Optimized Formulations

Table 11: R^2value of C-SLN.G5 in various kinetic models

Formulation	SLN.G5
Zero order	0.977
First order	0.774
Higuchi	0.989
Hixon Crowell's	0.970
Korsmeyer and peppas	0.987

Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model and showed zero order drug release with anomalous diffusion (Non Fickian diffusion) mechanism.

In-Vivo Studies of Optimized SLNs

In vivo anti Parkinsonism activity of the optimized formulations (SLN.G5) were performed by using Wister albino mice.

Pharmacodynamic Study

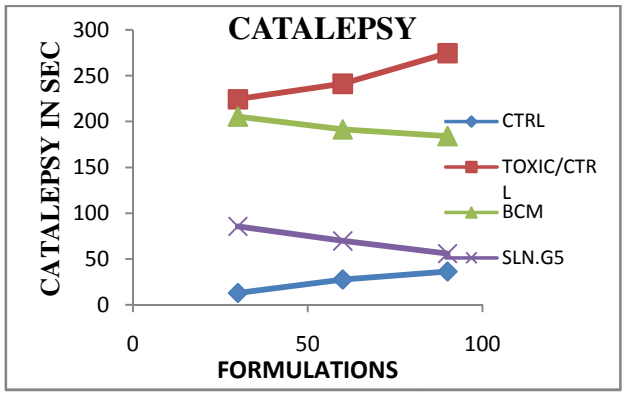

Figure 6: Catalepsy Behavior in Mice

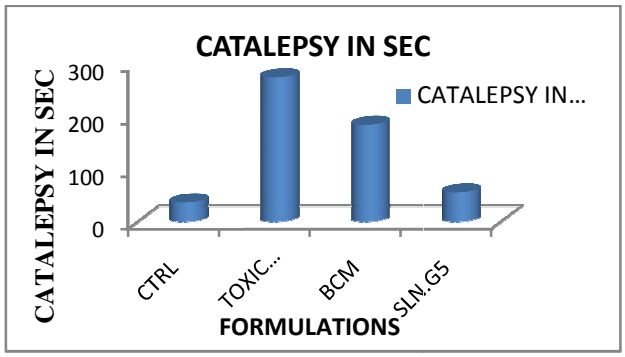

Figure 7: Catalepsy Behavior in Mice (at end of 120 min.)

Thus from the above results it was inferred that the optimized formulation SLN.G5 showed significant anti-catatonic effect in comparison with the standard. The cataleptic behavior of haloperidol treated mice was found to increase significantly after 120min of treatment ($p<0.05$) when compared to control treated mice. The Group-3 mice co-treated with bromocriptine solution intra peritoneal (i.p) showed a lower reversal catalepsy than Group-4. The Group-4 mice co-treated with BCM loaded SLN.G5 formulation significantly ($p<0.01$) prevented increase in catalepsy behavior after 120 min when treated with haloperidol treated mice. The Group-4 was nonstatistically significant from that of saline treated mice ($p>0.05$).

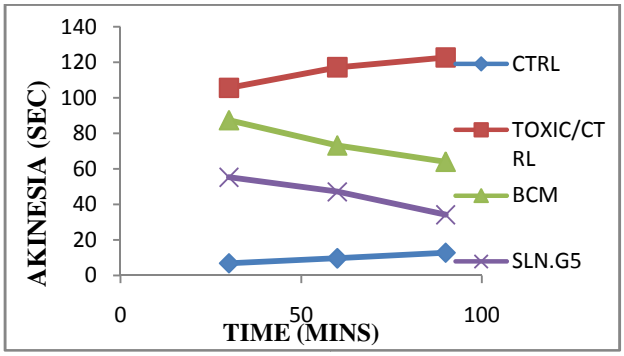

Figure 8: Akinesia Behavior in Mice

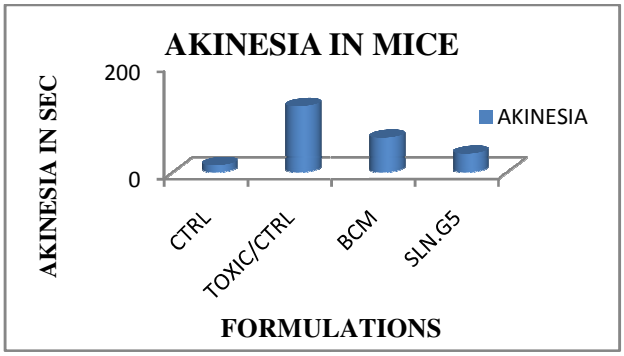

Figure 9: Akinesia Behavior in Mice (at end of 120 min.)

Thus from the above results it was inferred that the optimized formulation SLN.G5 showed significant akinesia effect in comparison with the standard. The pharmacokinetic study of optimized (SLN.G5) Bromocriptine mesylate Solid lipid nanoparticles showed most effective in reversing the cataleptic and akinesia state and restoring normal behavior. The mice co-treated with BCM solution and haloperidol administered intra peritoneal (i.p) and Group-4 showed akinesia score for 64±1.62 and 45.4±1.48 at 120s respectively. The Group-4 was nonstatistically significant ($p>0.05$) from that of BCM solution

and haloperidol treated mice. The Group-4 was able to reverse the cataleptic and akinesia score significantly ($p<0.01$) more compared to other formulations.

Biochemical Estimation

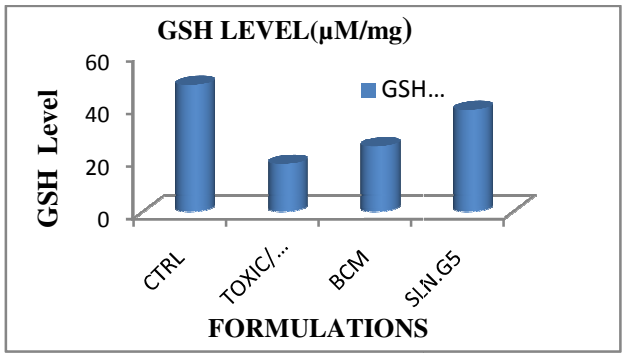

Figure 10: GSH Level in Mice

The results illustrated that mice co-treated with bromocriptine loaded optimized SLNs (SLN.G5) and haloperidol showed significantly higher GSH levels as compared to haloperidol induced mice. The GSH concentration was significantly lower in Group-2 as compared to Group-1 ($p<0.01$), Group-3 and Group-4 shows statistically nonsignificant ($p>0.05$) result among each other but relatively significant result as compare to saline and haloperidol induced mice ($p<0.05$). Bromocriptine loaded optimized SLNs (SLN.G5) treated mice showed significantly lower MDA content as compared to haloperidol treated mice. Haloperidol treated mice showed highly significant increase in MDA content as compared to saline treated mice ($p<0.01$). Group-3 and Group-4 results small decrease in MDA content, shows in non significant result ($p>0.05$) among each other but, relatively significant result as compared to saline ($p<0.05$). The Group-4 formulation showed significantly lower MDA content as compared to haloperidol treated mice ($p<0.01$).

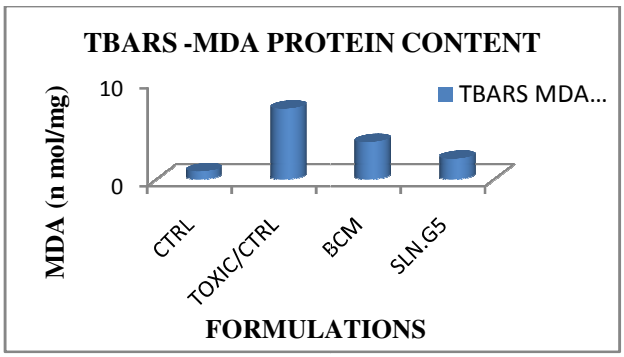

Figure 11: GSH Level in Mice

Stability Studies

The optimized formulations (SLN.G5) subjected to stability studies and shown in table 12.No significant changes in, entrapment efficiency, drug content and *in vitro* drug release were observed at storage condition of4±2°C,Room temperature and analyzed after the end of 1,2 and 3 months. From the results, it was confirmed that the SLN formulation had long-term stability; this could be attributed to higher solubility of drug in the lipid matrix and due to Pluronic F-68, and because of its nonionic nature it decreases the electrostatic repulsions between the particles, thus stabilizing the nanoparticles by forming a coat around their surfaces. The formulations were found most stable at refrigeration temperature and room temperature. No significant changes in Physical appearance, Entrapment efficiency, Drug content and *in vitro* drug release was observed. At 40°±2°C at 70±5% RH, there is no change in Physical appearance, Entrapment efficiency, Drug content and *in vitro* drug release are gradually decreased.

Table 12: Stability data for Optimized Formulation

Stability condition		Physical appearance				Entrapment Efficiency (%)				Drug content (% w/w)				*In vitro* Drug Release			
		Initial	**1**	**2**	**3**	**Initial**	**1**	**2**	**3**	**Initial**	**1**	**2**	**3**	**Initial**	**1**	**2**	**3**
Refrigeration 4±2°C	G5	NC	NC	NC	NC	90.94	90.72	89.99	88.53	98.92	97.98	96.55	94.35	98.75	98.09	96.66	94.85
Room Temp	G5	NC	NC	NC	NC	90.94	87.81	83.45	78.36	98.92	96.53	92.89	89.99	98.75	96.87	93.01	91.25
40±2°/ 70±5%RH	G5	NC	NC	NC	NC	90.94	84.18	76.18	71.82	98.92	92.17	83.45	79.09	98.75	95.93	91.54	88.94

*NC-No change

CONCLUSION

A SLN formulation has been developed aimed to overcome the problems of poor oral bioavailability of BCM and improve its therapeutic effectiveness. A low dose of haloperidol was able to induce oxidative stress and behavioral deficits that resemble PD very closely. The resulting SLNs effectively enhanced

the antioxidant effects of BCM. These encouraging results make the novel effective drug delivery system of BCM for the treatment of PD. However, more pre-clinical and clinical studies should also be performed in near future to establish these formulations in the market on the basis of low risk/high benefit ratio as compared to high risk/low benefit ratio in their present forms.

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