



## Targeting dopamine liposome to brain using polysorbate 80 for parkinson disease

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### Abstract

The aim of the present study was to prepare and evaluate Dopamine (Dp) liposome using polysorbate 80 (PS80) which enabled the targeted delivery of drug across Blood Brain Barrier (BBB) in the effective treatment of Parkinson disease as L dopa drug conventionally used for Parkinson's disease produces "wearing off" adverse reactions. Dp loaded liposome were prepared with various concentration of PS80 by Reverse Phase Evaporation technique. The formation of liposome was confirmed by scanning electron microscopy (SEM). The drug and the excipient compatibility was analysed by FTIR, DSC and XRD studies. FTIR indicated that there was no loss in chemical integrity of the drug upon fabrication into liposome. DSC and XRD results demonstrated that the drug was changed from the crystalline form to the amorphous form in the formulation. Among all the formulations, LSP4 showed higher Entrapment efficiency (EE) of 68% and increases the animal locomotion in psychopharmacological study (18.3%). Dp Liposomal formulation LSP4 could be a choice for dopamine delivery directly to brain for Parkinson treatment alternative to conventional use of L dopa thus preventing "wearing of effect."

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## Introduction

Parkinson disease is an age related disease resulting in loss of dopamine leading to motor neurons malfunction causing extrapyramidal side effects such as bradykinesia, tremor and rigidity (Poewe *et al.*, 1998). L dopa is currently the most effective and conventional treatment for parkinson disease and is a prodrug of dopamine hydrochloride (Dp) which is given as 250mg oral per day. L-dopa is converted to dopamine after crossing the blood brain barrier (BBB) and its activation of central dopamine receptors(D2) improves the symptoms of Parkinsonism disease(Hefti *et al.*, 1981). However, long term, L dopa therapy results in motor complications such as motor fluctuations and dyskinesia called “wearing effect” or “on or off” response to patients (Quinn *et al.*, 1998;Schrag *et al.*, 2000 ). At “on” state the patient experiences mobility and “off” state they are unable to move their joints or immobility (Granerus *et al.*, 1978).

As an alternate to conventional L dopa for preventing its “wearing off “effect”, Dp can be given by brain targeted drug delivery. Dp is an essential agent used to manage parkinsonism, but does not cross the BBB and has a very short biological half life (Jain *et al.*, 1998). The lipid nature and the vesicular nature of the liposome, which can be a good source for designing brain targeted drug deliveries increases the BBB penetrability through endothelial cell membranes (Burkhard schlosshauer *et al.*, 2002) and early researches had showed polysorbate 80, a P- glyco protein inhibitor has the ability for transporting carrier loaded drugs across BBB after administration using many drugs like doxorubicin, tubocurarine by P-glyco protein inhibition (Alvautdin *et al.*, 1998). The aim of the present study was to prepare and evaluate Dp liposome using polysorbate 80 which enabled the targeted delivery of drugs across BBB in the effective treatment of Parkinson disease.

## Materials and methods

Polysorbate80, Span80, Haloperidol and Dopamine Hydrochloride (Dp) were purchased from Indian Research Products, India. Cholesterol (Ch) and

lecithin soya phosphotidyl choline (Lec) were purchased from Himedia Laboratories. All other ingredients used were of analytical grades.

### Preparation of liposome

Drug loaded liposome was formulated by reverse phase evaporation technique (Szoka *et al.*, 1978). The lipids - soy lecithin and cholesterol (9:1) were dissolved in diethyl ether. Dp (2mg/ml) in Phosphate buffer Solution (PBS) (pH 7.4) was added to this lipid solution along with polysorbate 80 (PS80) at molar concentration (Table 1). As the surfactant PS80 alone does not favour liposome formation, span 80 was also included at varying molar concentration to aid the formation of liposomes (Table 1). The formulation was homogenised (homogeniser, konteglass co. Vineland ,NJ) at 5000 rpm for 20 minutes at 50°C and cooled, a semisolid gel is formed. This gel was converted to a fluid consistency by mechanical agitation using a vortex mixer. To hydrate the vesicles, PBS was added to produce a suspension of multilamellar vesicle (MLV). The MLV was then sonicated (Microtip probe sonicator) for 30 minutes at 40% frequency to produce a homogeneous liposome. Liposome with only span 80 was prepared for comparison (LS 80) and liposome without surfactant was prepared for control.

### Entrapment efficiency (EE)

Minicolumn centrifugation method was carried out to determine the EE of the liposomal formulation (Fry *et al.*, 1978). Dp liposomal suspension was slowly added (100 microlitre) on sephadex G50 column and centrifuged at 3000 rpm for 10 minutes. The eluted liposome was then disrupted using diethyl ether and percentage entrapment can be quantified spectrophotometrically (Maghraby *et al.*, 1991) using the formula given below (Table 1, Fig. 1.)

### Psychopharmacology studies

Locomotor activity: Wistar albino rats (100-150 gm) were purchased from king institute, Chennai, India. They were acclimated to the institutional animal house condition, fed with food and water ad libitum. The experimental observation was made between

10.00 am and 16.00 pm hours in a quiet room at 23°C -25°C. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA. L dopa is used as a standard drug. Haloperidol is used to induce Parkinson disease producing extrapyramidal side effect and reduces locomotion of the animal. Dopamine was the most potent inhibiting the binding of haloperidol over dopamine receptor and increases the animal locomotion (Philip Seeman *et al.*, 2006).

Locomotor activity was measured by photo beam interruption in a digital actophotometer (Kulkarani *et al.*, 1999). The animals were grouped in to 11 groups of six each and each animal was placed in the actophotometer for 10 minutes to determine the basal normal locomotive movement by total photo beam interruptions. Group 1 does not receive any drug or formulation and serves as a control. Haloperidol (1mg/kg body weight) was administered intraperitoneally for inducing drug induced parkinsonism to all the groups except group 1. After 15 minutes, group 3 received Ldopa (10 mg/Kg body weight) and the formulation were given intraperitoneally (8 mg/kg body weight) to all other groups (table 2). The locomotor activity from its basal readings was observed for 10 minutes at regular interval of 0,30, 60, 90 and 120 minutes.

#### *Statistical analysis*

All the experiments were done six times and the resulting data were expressed as mean  $\pm$  standard deviation (SD) and Tukey's *post-hoc* test was also done to analyze if the significant difference between different groups using the statistical analysing software SAS (version 9.1.3 sp4 portable).

#### *X-ray diffraction studies*

X-ray diffraction (XRD) patterns were recorded for the formulation and Drug Dp by DRON-2 powder diffractometer (CuK $\alpha_1$ ,  $2$  radiation,  $\Delta 2\theta = 20^\circ\text{C}$ -140°C). The liposomal formulation was lyophilized before the XRD studies. LSP4 was selected on the basis of its optimum response on psychopharmacological studies. The XRD pattern for

the pure Dp, Physical mixture of liposomal constituents and LSP4 were determined.

#### *FTIR study*

FTIR study by disc method was done to find any drug excipients interaction (Robert, *et al.*, 1970). Dp and Dp with excipients were prepared in Potassium Bromide disks and subjected to FTIR studies using FTIR spectrophotometer (Perkin Elmer Spectrum one). The FTIR spectrum of the formulation was then analyzed at scanning range of 450 cm $^{-1}$  to 4000 cm $^{-1}$  and comparison with the spectrum of standard Dp for any drug degradation

#### *Differential Scanning Calorimetry (DSC)*

Thermograms of Drug - Dp, Physical Mixture and LSP4 were recorded in Differential Scanning calorimeter (Spectrum one, Perkin Elmer, Model Sd10). About 2mg of each sample were heated separately in a sealed aluminium pan (capacity - 4 $\mu$ l) under nitrogen flow (30ml/minute) over the temperature range 25°C - 250°C with a heating rate of 10°C/min.

#### *Scanning electron microscopy (SEM)*

Surface morphology of surfactant modified liposomal formulations was studied by Scanning Electron Microscope (JEOL Electron Microscope, Japan). A thin layer of sample were fixed on the carbon adhesive tape on an aluminium stub. The scanning electron micrographs of the sample were obtained by random scanning of the stub

## **Results and discussion**

#### *Entrapment Efficiency*

The Entrapment efficiency of the formulation increases with increase in concentration of PS80. Among all the formulation, LSP4 had highest EE (68.7%). When the concentration of span 80 increases, there is no appreciable changes in the EE (Table 1 and Fig. 1).

#### *Psychopharmacology studies*

The percentage change or rise of locomotor activity from its normal value was given in Table 2 and Fig. 2.

Group 1 animals showed no marked change from its basal normal values as it received only PBS. Group 2 received only haloperidol and it does not show any appreciable rise in locomotor activity and showed larger deviation from the normal value (91.2%) Hence haloperidol restricts the animal locomotion producing

drug induced parkinsonism. As per the results, Dp in the liposomal formulation increases the locomotion within 2 hours. Among all the liposomal formulation, group 8 (LSP4) showed an appreciable rise in locomotion (18.3%) which was comparable to group 3(10.2%) with standard drug Ldopa.

**Table 1.** Composition, Formulation code and Entrapment Efficiency of the formulation.

Formulation	Formulation code	Lipid surfactant composition (Molar ratios)	Entrapment efficiency*
Le:Ch	NS	9:1	32.62±0.34
Le:Ch:Span80	LS80	9:1:1	46.85±0.57
Le:Ch:Span80:Polysorbate80	LSP 1	9:1:1:1	61.45±0.66
Le:Ch:Span80:Polysorbate80	LSP 2	9:1:1:2	66.53±0.58
Le:Ch:Span80:Polysorbate80	LSP 3	9:1:2:1	59.37±0.64
Le:Ch:Span80:Polysorbate80	LSP 4	9:1:1:3	68.74±0.91
Le:Ch:Span80:Polysorbate80	LSP 5	9:1:3:1	34.65±0.68
Le:Ch:Span80:Polysorbate80	LSP 6	9:1:1:4	72.65±0.34
Le:Ch:Span80:Polysorbate80	LSP 7	9:1:4:1	42.38±0.29

Le - Lecithin, Ch - Cholesterol (Lecithin and cholesterol were used to formulate liposomes), \*(n=6 ± S.D.) p<0.01.

**Table 2.** Percentage of reduction in Locomotor activity in response to the formulations.

Group	Formulation /control		Time interval for evaluation			
			30 minutes	60 minutes	90 minutes	120 minutes
			Percentage of reduction in locomotor activity from its basal/normal values			
1	PBS	Percentage of reduction in Locomotor activity	5.31 ± 1.32	6.81 ± 2.05	4.31 ± 2.02	5.11 ± 3.88
2	H		96.64± 0.84**	95.24± 0.56**	92.67± 0.62**	91.22± 2.27**
3	H+Ldp		43.42± 1.03*	21.51± 1.62*	18.52± 1.32*	10.24± 1.54*
4	H+LS80		88.26± 1.06**	81.33± 0.53*	74.37± 2.06**	70.52± 2.55**
5	H+LSP 1		84.91± 0.77**	80.21± 0.62**	78.25± 1.98**	75.26± 0.82
6	H+LSP 2		64.35± 0.83*	56.21± 1.54*	46.32± 1.12*	36.83± 1.22*
7	H+LSP 3		94.21± 2.04**	90.33± 1.59*	87.65± 0.58**	84.63± 2.28**
8	H+LSP 4		53.61± 2.08**	32.83± 0.26**	23.41± 2.65*	18.32± 1.66*
9	H+LSP 5		96.53± 1.52*	96.85± 0.82**	88.35± 1.64**	84.38± 0.53*
10	H+LSP 6		92.33± 2.21**	89.22± 1.82**	84.45± 1.55*	82.62± 1.41*
11	H+LSP 7		96.61± 0.85*	91.27± 2.27*	87.32± 1.85**	84.55± 1.92**

PBS- Phosphate buffer saline, H- Haloperidol, Ldp - Levo dopa (L dopa).

All groups received haloperidol except group 1. Group 3 to group 11 received respective formulations after 15 mins of haloperidol administration.

Values are expressed as mean ± SD six animals in each group, comparison were made between group 1 Versus group 2 to group 11 (\*p<0.01, \*\*p<0.001).

This might be due to the competitive binding of Dp replacing haloperidol in the dopamine (D2) receptor and this lessens the effect of haloperidol inhibiting locomotor activity of LSP4 (Kulkarni *et al.*, 1999). This causes low percentage of reduction of locomotor

activity for LSP4 than other test formulations. Thus, PS80 was in concordance with the early studies for its efficient BBB penetration enhancer promoting flexibilities to the vesicles ferrying dopamine to brain (Alyautdin *et al.*, 1998).

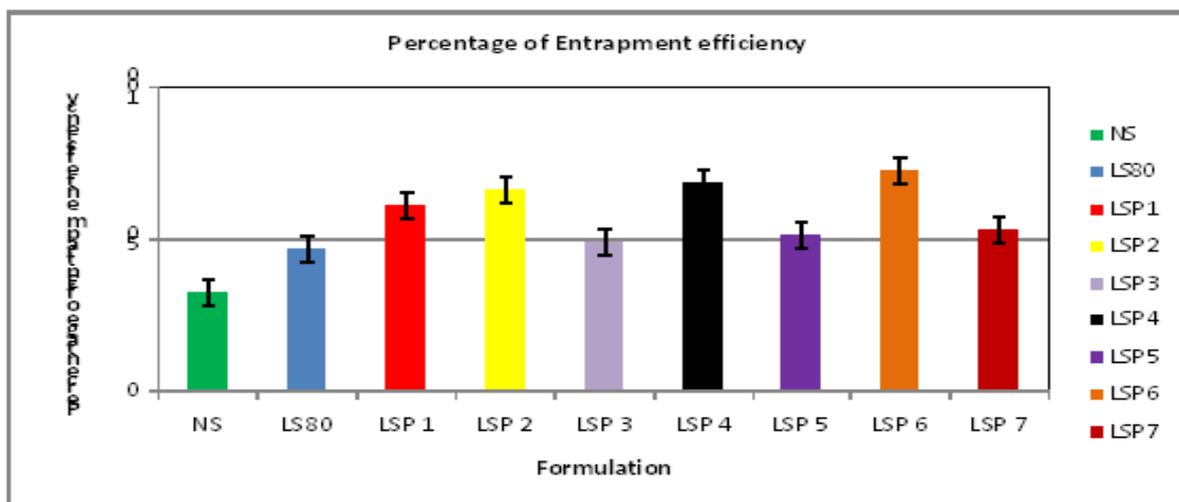
**Table 3.** Characteristic IR Absorption Peak of Drug Dp and DP with excipients.

Dopamine (Dp) Peak (cm <sup>-1</sup> )	Absorption Physical mixture (cm <sup>-1</sup> )	Absorption Peak vibrations (cm <sup>-1</sup> )	Absorption range for stretching(θ) Functional Group
3423.7	3435.8	3410 - 3440	O-H
3103.2	3148.4	3015 - 3440	N=H
1643.6	1644.8	1640 - 1645	NH <sub>3</sub>
848.5	853.4	840 - 855	C-H

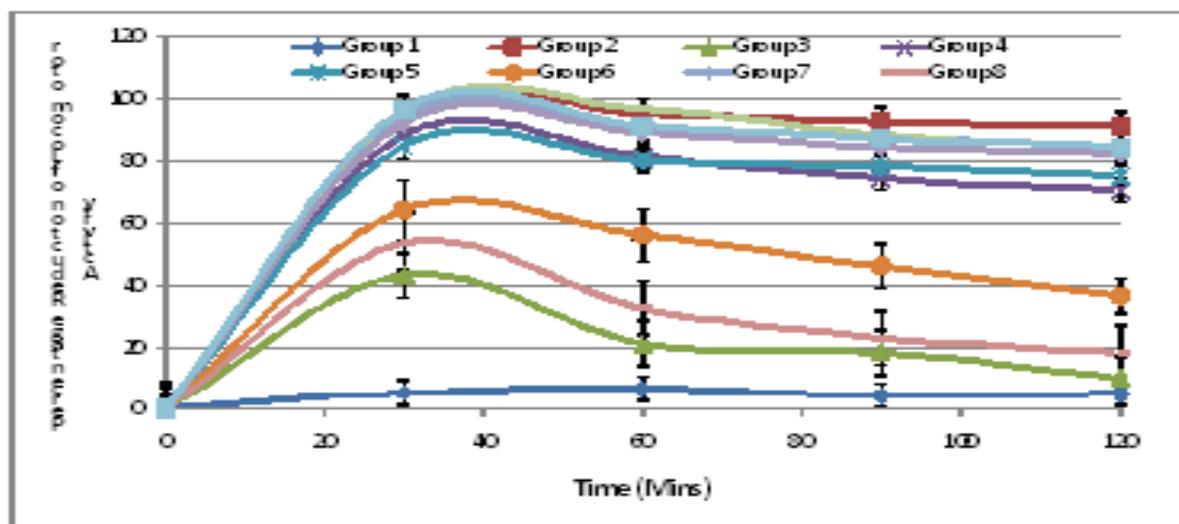
*X Ray Diffraction studies (XRD)*

Samples of formulation constituents (Physical mixtures) and the drug Dp were analysed for XRD. The XRD analysis (Fig. 3) revealed that the crystallinity of Dp drug was lost during the liposomal formulation. The large, sharp peaks of drug at 19.8,

23.4 and 27.4 signify crystalline regions of the drug which were absent in the liposomal formulation. This proves that the drug which was available in the formulation was highly dispersed or dissolved in the liposomal formulation as amorphous form.



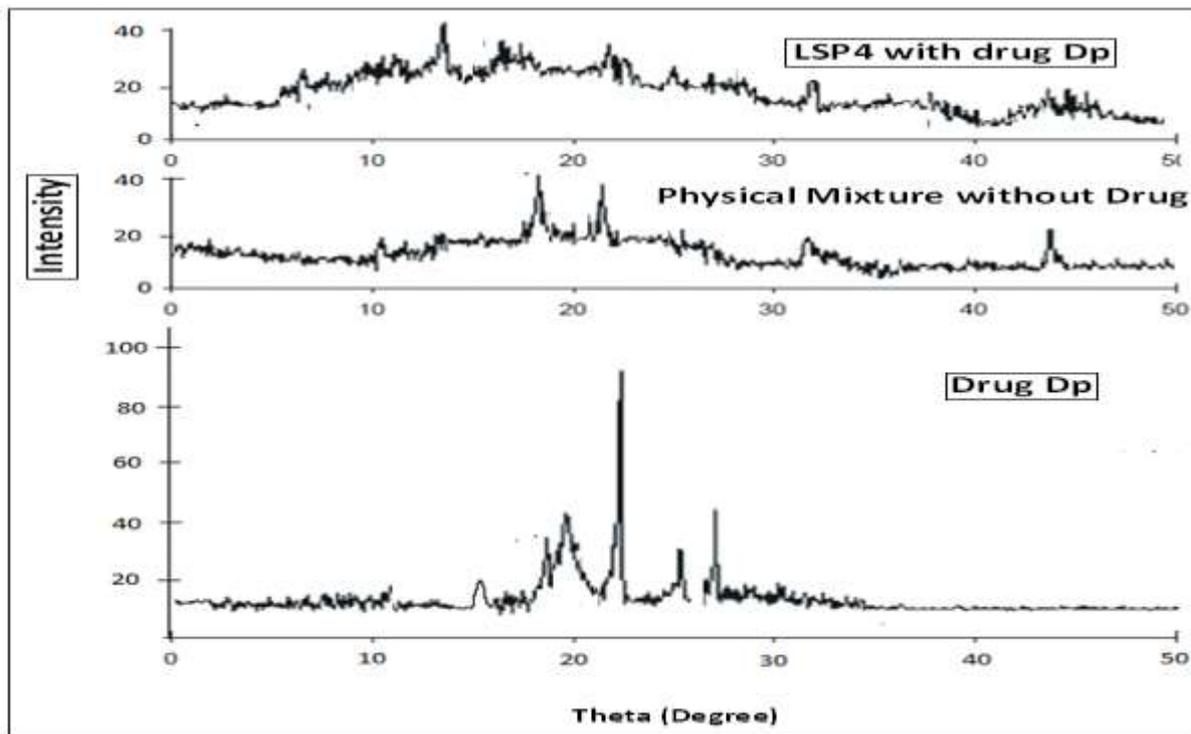
**Fig. 1.** Entrapment efficiency of surfactant liposomal formulation in comparison with the control liposome (without surfactant-NS).



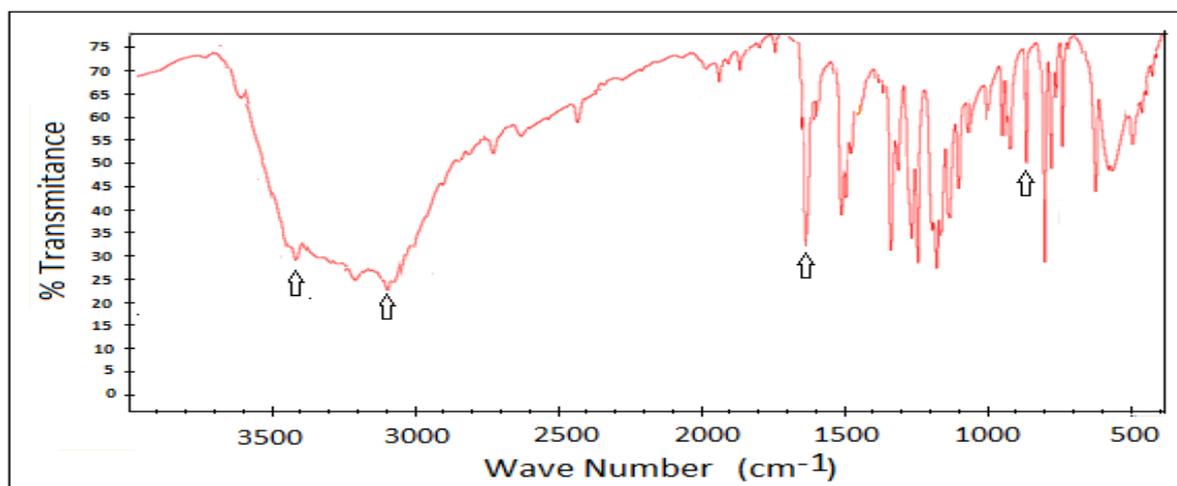
**Fig. 2.** Percentage of reduction of Locomotor activity in response to the formulation in antagonizing haloperidol reduced locomotor effect.

*Fourier Transform Infra-Red Spectroscopy (FTIR)*  
FTIR spectrum (Fig. 4) of Dp displays characteristic absorption peak (Fig. 6 and Table 3) for the OH, NH, CH, amine group stretching (9) and bending

vibration. These peaks were also present in the physical mixture (Fig. 5) indicating no interactions between the drug and the excipients.



**Fig. 3.** XRD studies of the formulation.



**Fig. 4.** Pure Drug - Dp FTIR Spectrum.

#### *Differential Scanning Calorimetry (DSC)*

The endothermic peak for the drug was found in the range of 147°C – 150°C in drug and physical mixture sample (Fig. 7), which was absent in the LSP4 formulation indicating absence of drug crystallinity, due to complete solubility of the drug in the vesicular

layer. In the physical mixture sample, cholesterol peak was prominent at 148.4°C and for lecithin at 225.3°C. The broad peak of LSP4 between 50°C to 100°C was due to the hydration of the vesicles (Hamdy Abdelkader *et al.*, 2007).

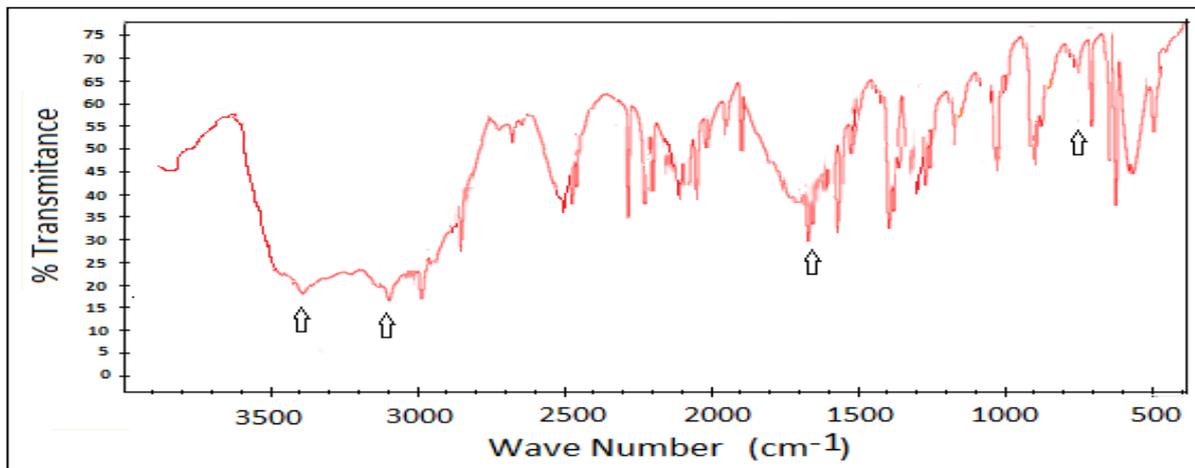


Fig. 5. FTIR Spectrum of Drug - Dp with excipients (Physical mixture).

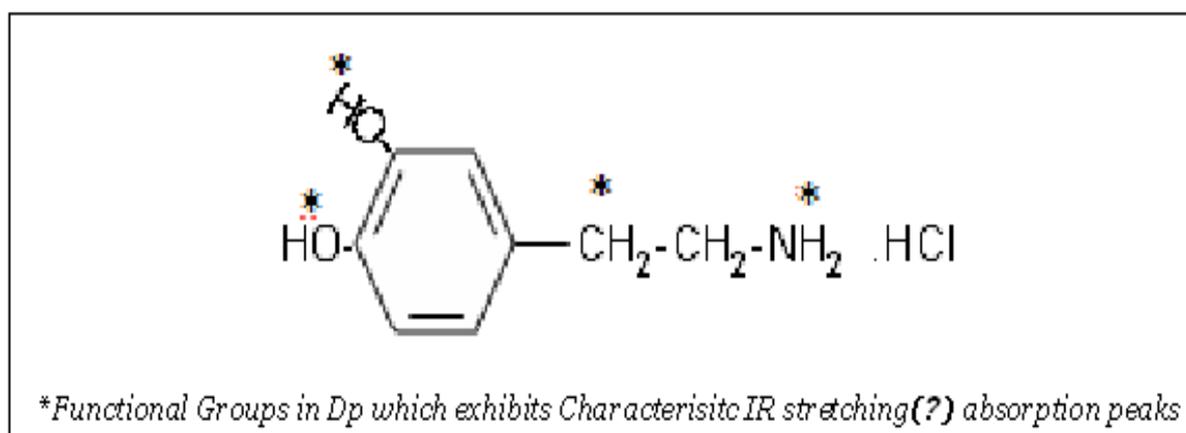


Fig. 6. Structure of Dopamine (Dp).

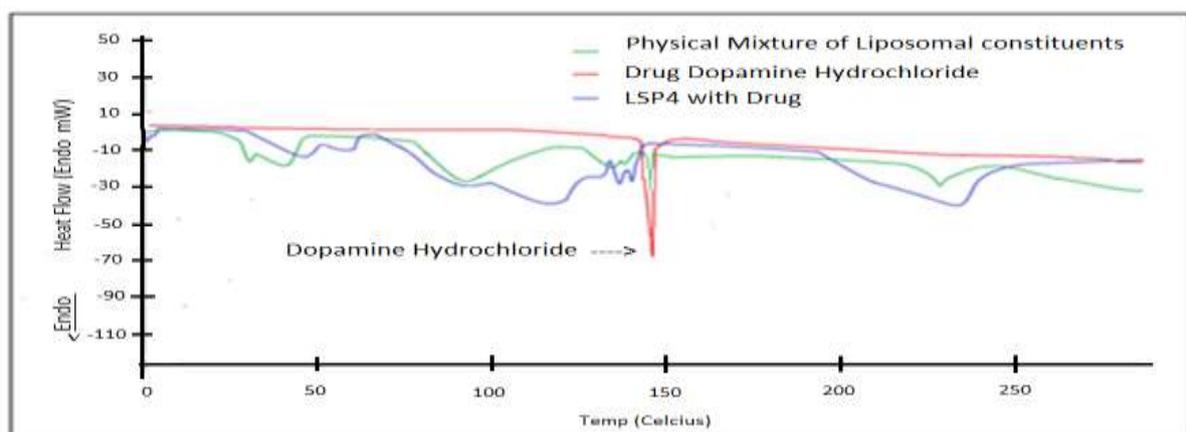
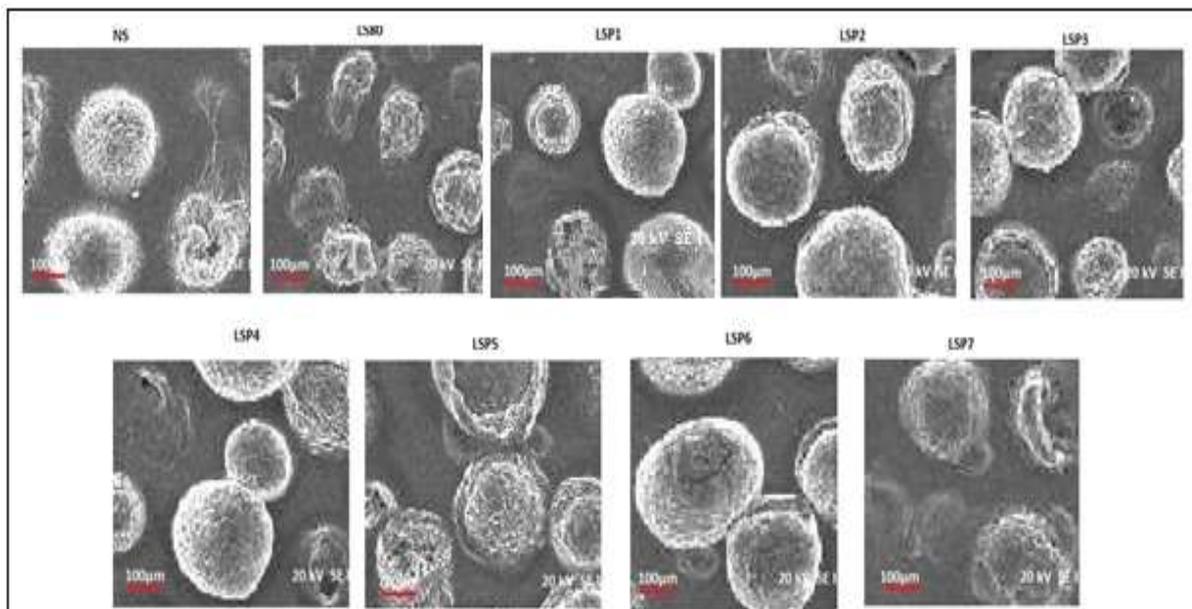


Fig. 7. DSC of the formulation LSP4, Drug dopamine Hydrochloride and physical mixtures.

#### Scanning Electron Microscopy (SEM)

All the formulation showed a prominent spherical structure (Fig. 8) ensuring liposomal formation. Inclusion of PS 80 with span 80 produces the sphericity of the liposomes indicated by the SEM of

LSP1 to LSP7. LS80 devoids the special nature and might be due to absent of PS 80. Absence of any undissolved drug crystals also indicates the inclusion of drug in the liposomes.



**Fig. 8.** SEM Photograph of the formulations.

### Conclusion

Drug loaded liposome was prepared with varying concentration of PS80 using REV technique. Among all the formulation, LSP4 showed higher EE (68%). Psychopharmacological study showed haloperidol induced Parkinsonism was controlled by LSP4 which increases the animal locomotion. SEM studies confirmed the spherical nature of liposome. XRD studies revealed the loss of drug crystallinity as the drug had intercalated with the vesicles in amorphous form. FTIR and DSC study revealed the absence of drug interaction with the excipients. Thus, LSP4 could be a better choice for delivering Dp to brain for Parkinson therapy than conventional L dopa thus avoiding its “wearing off” effect.

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