RESEARCH ARTICLE

# Formulation and psychopharmacological evaluation of surfactant modified liposome for parkinsonism disease

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A lipid based drug delivery was designed for the treatment parkinsonism diseases by delivering neurotransmitter – dopamine directly in the brain. Surfactants – span 20 (S20), span 40 (S40), span 80 (S80) and span 80 - tween 80 combination (ST80) were used to prepare the carrier liposomes. Psycopharmacological studies were performed in wistar rats towards evaluating reduction of parkinsonism's extrapyramidal side effects using actophotometer and rotorod. It was found that ST80 formulation be the best in arresting the effect of haloperidol induced parkinsonism than the other formulation. In conclusion, administration of dopamine in ST80 modified liposomes could have considerable therapeutic effects making it to be an ideal carrier in ferrying the drug of choice direct in to the brain and thus proving a good candidate for parkinsonism treatment.

***Keywords:*** *Surfactant, Span 20, Span 40, Tween 80, Parkinsonism, Liposomes.*

## INTRODUCTION

Brain drug deliveries are the rate limiting step in the translation of progress in the molecular neuroscience into clinically effective neurotherapeutics for patient with disorders of the central nervous system (CNS). Many drugs are prevented in delivering them to brain because of their barriers. Some of the drugs that are prevented for entering the brain are antibiotics, anti cancer drugs and a great range of neuropeptides which can creates problem in treating brain disorder. Progress in brain drug delivery has lagged behind other areas in the molecular neurosciences, because of the difficulties posed by the blood brain barrier [1].

Parkinsonism diseases of brain are an age related diseases arising from the degeneration of dopaminergic nigro – striatal neurons of the basal ganglia resulting in decreased dopamine secretion. This causes various extra pyramidal side effects such as bradykinesia, tremor and rigidity [2]. The current therapy concentrates for symptomatic relief as no agent is capable of inhibiting the neuronal degeneration has yet been found. Levodopa (L-dopa), muscarinic acetyl choline receptor antagonists and monoamine oxidase inhibitors [3] are **t**he most commonly used agents**,** but prevalently L- dopa was used. Patients receiving long term levodopa therapy commonly experience a "wearing off effect" which means that the duration of benefit usually shortens from each dose of levodopa as the therapy progresses. Patients also have possibility of developing sudden unpredictable fluctuations between mobility and immobility characterized by "on – off phenomenon". In the "on state" the patients experiences normal mobility which is then followed by "the off state" wherein the patient may be unable to rise from the chair on which he had sat down a few minutes ago. Levodopa also has a very short half life (1- 2 hrs) which causes such fluctuations in its plasma concentration [4].

The direct injections of the neurotransmitter-dopamine in to the brain parenchyma cannot offer adequate long term treatment of these neurodegenerative diseases [5]. Therefore dopamine neurotransmitter drug can be potentially loaded in a carrier for delivering safely in to the brain which may result in treatment of parkinson's disease. Colloidal drug carriers which include micelles, emulsions, lipos-

omes, nanospheres and nanocapsules can be made as targeting carriers to brain for delivering the neurotransmitters for parkinsonism therapy. These colloidal carriers can allow access across the BBB (Blood Brain Barrier) of non transportable drugs by masking their physio-chemical characteristics through their encapsulation [6].

Liposome - one of the best and suitable colloidal drug carrier described by bangham [7] is a lipid based microscopic vesicles, having concentric lipid bilayers and are separated by water or aqueous buffer compartments with diameter ranging from 80 nm to 10 micron. This liposome, as a drug delivery possesses the potential for providing controlled release of administered drugs and increases the stability of labile drugs [8]**.** Encapsulation of drugs in to liposomes produces a novel approach to sustain the drug and protein release [9]. Levin demonstrated that there is a positive correlation between the lipophilicity of 27 selected compounds and their ability to cross BBB [10]. Peptides and proteins must be modified to increase their permeation across the BBB. This can be achieved by increasing the lipophilicity of the protein and thus enhances its ability to penetrate the endothelial cell membrane and cross the BBB [11]. Thus lipidicity of the drug (either by drug modification or lipid carrier) has a strong positive correlation towards blood brain barrier drug delivery. Liposome carrier which is a lipid based drug delivery can be considered for targeting brain. In the presence of suitable surfactants, the brain delivery of drugs can still more enhanced which may do so by increasing the structural flexibility of the carrier helping the carrier to squeeze through the barrier. Experiment with such surfactants using tail flick test and dalargin as experimental drug showed that polysorbate 20, 40 and 60 were able to transport dalargin to brain and produced an antinoceptive effect [11]. Thus a lipid based drug carrier with suitable surfactant can be a potential nanoparticle facilitated drug delivery to brain.

The aim of our study was to prepare liposome entrapping dopamine hydrochloride with the aid of various non ionic surfactants such as span 20, span 40 and span 80 and comparing their ability to deliver the antiparkinsonism drug - dopamine hydrochloride in reducing the haloperidol induced extra pyramidal side effects of parkinsonism in animal model using psychopharmacological studies. Those carriers were subsequently characterized for their differential scanning calorimetry (DSC) for drug stability with the formulating excipients and *in vitro* release studies to evaluate the release characteristics of the drug from the formulation.

## MATERIALS AND METHODS

### Materials

Cholesterol (Ch) and lecithin soya phosphotidyl choline (Lec) were purchased from Himedia Laboratories Pvt. Ltd., India. Diethyl ether was obtained from Paxmy Speciality Chemicals, India. Surfactants - Span 20, Tween 80, Span 40 and Span 80 were purchased from Indian Research Products, India. Haloperidol was obtained from Mano Pharmaceuticals, India. All other ingredients used were of analytical grades. Rats (wistar albino rats, male, 7 weeks old and approximately 150 g body weight) were used for the study.

### Methods

### Formulation of liposomes

Liposomes were prepared by using lecithin (Lec) and cholesterol (Ch) with some modification of "Reverse Phase Evaporation Technique" as described by Szoka and Papahadjoupoulos et al [13]. Multi lamellar vesicles liposomal formulation were prepared by using lecithin and cholesterol with different non ionic surfactants such as span 20 , span 40 , span 80 and tween 80 entrapping the drug dopamine hydrochloride (Dopamine HCl). In brief, lecithin and cholesterol (9:1) were solubilized in diethyl ether, then 5 ml of dopamine HCl drug (2 mg/ml) in phosphate buffer saline (PBS) solution of pH 7.4 were added along with surfactant span 20 , span 40 ,span 80 separately at molar concentration (Table 1) of 9:1:1(Le:Ch:surfactant).

**Table 1. Composition of the formulation**

| Formulation | Formulation Code | Lipid composition (Molar Ratio) |
|---|---|---|
| Le:Ch:Span 20 | S20 | 9:1:1 |
| Le:Ch:Span 40 | S40 | 9:1:1 |
| Le:Ch:Span 80 | S80 | 9:1:1 |
| Le:Ch: Span 80: Tween 80 | ST 80 | 9:1:2:1 |
| Le:Ch | NS | 9:1 |

As tween 80, does not favour vesicle formation, combination of span 80 and tween 80 were tried at molar concentration of 9:1:2:1(Lec: Ch: Span 80:

tween 80). The optimized molar ratios of lipid and surfactant were selected which was based on their stability and vesicle formation in PBS pH 7.4 followed by addition of vitamin E at 0.6 mol% for stability. The formulation was emulsified by high shear mixer using a homogenizer (Tenbroeck tissue grinder - Kontes Glass Co., Vineland, NJ) at 5000 rpm for 20 minutes at a temperature of 50°C. The dispersion was cooled to room temperature and the weight of the dispersion was adjusted before swelling. This led to the formation of a reverse type (w/o) emulsion which later forms a semi solid gel like consistency. The residual diethyl ether solvent is evaporated further by using vacuum evaporator (BUCHI EL 131 Rotavapor, Germany) under reduced pressure (260 - 400 mm Hg) at 60°C. The lipid gel so formed was collapsed and transformed in to a fluid consistency by continuous and vigorous mechanical agitation using a vortex mixer (REAX top, Heidolph Instruments GmbH & Co., Schwabach, Germany). To this 5 ml of warm phosphate buffer saline (pH 7.4) was added to hydrate the vesicles thus producing suspension of multi lamellar vesicle liposomes (MLV). The resulting liposomes were then sonicated using a microtip probe sonicator (Vibracell, sonics and materials, Inc, Danbury, CT) for 30 minutes at 40% frequency to produce a complete homogeneous dispersion. The preparation was then flushed with nitrogen gas for 1 minute to remove dissolved oxygen and to ensure complete removal of all traces of organic solvent. The weight of the liposomes was adjusted to the initial weight prior to sonication. The liposome dispersion samples were kept at 4°C and protected from light .Prior to use, they were filtered through whatman filter paper No 42 (pore size 2.5 μm). The formulation prepared using span 20, span 40, span 80 and combination of span80 – tween 80 was coded as S20, S40, S80 and ST80 respectively for identification. The conventional drug containing liposomes (lecithin soya phosphotidyl choline: cholesterol) that do not have surfactants were prepared by the same method in absence of the surfactant as described in above procedure entrapping the drug and was coded as NS.

**Purification of liposomes**

The unentrapped drug and traces of residual solvents from the samples were removed by dialyzing process [14]. In brief, the liposomal formulation were dialyzed against PBS (pH 7.4) solution overnight (12 hours) using cellulose membrane tubing (spetra/por, spectrum Medical Industries , New Brunswick, NJ, USA) with molecular cutoff at 8000 - 12000 which was previously stored in PBS solution before use. The system was maintained at 300°C by means of a circulating water bath. The dialyzing solution was continuously stirred with a magnetic stirrer and changed six times during dialysis for 24 hour. The system was protected from light by wrapping with aluminum foil throughout.

**Fourier transform infra red spectroscopy (FTIR)**

In order to get evidence on the possible interaction of drug with excipients of the carrier at molecular level, FTIR study by disc method was carried**.** Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) and subjected to FTIR studies using Perkin Elmer Spectrum one, FTIR spectrophotometer (1600 series, Perkin-Elmer Inc, Norwalk, CT). The scanning range was from 450 $cm^{-1}$ to 4000 $cm^{-1}$ and the resolution was 1 $cm^{-1}$. The FTIR spectrum of the formulation was then analyzed in comparison with the spectrum of standard dopamine HCl for evidence of any drug degradation [15].

***In vitro* release studies**

The release studies of the formulation S20, S40, S80, ST80 and NS were carried out in a 250 ml beaker containing 100 ml of PBS medium for 36 hours [16] .The medium was stirred using a magnetic needle at 50 rpm. Dialysis membrane (Hi-media) was used as a barrier to isolate the donor and the receptor phase. PBS (pH 7.4) was utilized as the medium for determining the release of dopamine HCl from the liposomes. PBS (100 ml) was placed in 250 ml beaker. Dialysis membrane measuring 2 inches was taken and one end of the membrane was sealed by close – fitting clip. The purified liposomal samples (without unentrapped drug) were filled in the dialysis bag and the other end was closed with another clip. The dialysis membrane containing the samples was suspended in the medium with the help of a Rheotort stand. Release studies were carried out at a temperature of 37 ± 0.5°C under mechanical magnetic stirring (Remi Equipments) at 50 rpm. At regular fixed time interval, samples were withdrawn from the PBS solution, filtered and determined

spectrophotmetrically at 280 nm (shimadzu, model 2100) after suitable dilution with PBS. Sink condition was maintained during the release experiments. The release data was then suitably analysed.

**Psychopharmacological study**

The psychopharmacological study was carried out for the test and control formulation [17]. wistar albino rats 100 – 150 g were purchased from King Institute, Chennai, India. They were acclimated to the institutional animal house conditions, fed with food and water ad libitum. The experimental observations were made between 10.00 and 16.00 hours in a quiet room at 23-25ºC. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA. The animals were then subjected to psychopharmacological evaluation for extra pyramidal side effects of parkinsonism using actophotometer for change in locomotor activity and rotorod for change in muscular coordination. Haloperidol was used for the induction of extra pyramidal effects (drug induced parkinsonism). L-dopa (Syndopa) was used as a standard drug. Remaining formulation has dopamine HCl in surfactant modified or unmodified liposome.

**Locomotor activity**

The locomotor activity (horizontal activity) was measured using a digital actophotometer (Space-lab, India). The animals were weighed, numbered and grouped in eight groups of six animals. The animals were individually placed in the actophotometer for 10 minutes and basal locomotor activity (extra pyramidal effect) was determined for all the animals. The locomotor activity was expressed in terms of total photo beam interruption for ten minutes of time duration. After estimation of basal activity, the groups were then labeled as group 1, group 2, group 3, group 4, group 5, group 6, group 7 and group 8. PBS (1 ml) solution containing drug dopamine hydrochloride with physical mixture of surfactant (span20, 40, 80 and tween 80) were administered to group 1 animals, haloperidol (1 mg/kg body weight) were administered to groups 1, 2, 3, 4, 5, 6, 7 and 8. After 15 minutes, group 3 animals received L-dopa (Syndopa) (10 mg/kg body weight) as control , group 4 received S20 (8 mg/kg body weight) test formulation, group 5 received S40 test formulation, group 6 received S80 test formulation , group 7 received ST80 test formulation and group 8 received NS (non surfactant) formulation. All the control and test samples were administered to animals by intraperitoneal injection. The concentration of drug dopamine HCl in the formulation was 8 mg/kg body weight of locomotor activity (extra pyramidal effect) from its basal readings was observed for 10 minutes at regular intervals of 0, 30, 60, 90 and 120 minutes.

**Muscular coordination activity**

The motor coordination was assessed using digital rotorod apparatus (Inco- Ambala, India). In this experiment, rats were trained by placing them on a rotating rod (20 rpm), twice daily for three consecutive days before the experiment. An interval of 30 minute was kept between two trails. Only those rats which have demonstrated their ability to remain on the rotating rod for at least 2 minutes were selected and used for the experiment**.** These selected rats were divided into eight groups with 6 animals in each group. The rats were then tested for motor coordination to record basal "fall off" time. After estimation of basal "fall off" time, PBS solution was administered intra peritoneally to group 1 animals, haloperidol (1 mg/kg body weight) was administered intra peritoneally to group 2, 3, 4, 5, 6, 7 and 8. After 15 minutes, group 3 animals received L-Dopa (Syndopa) (10 mg/kg body weight) control, group 4 received S20 (8 mg/kg body weight) test formulation, group 5 received S40 (8 mg/kg body weight) test formulation, group 6 received S80 (8 mg/kg body weight) test formulation, group 7 received ST80 (8 mg/kg body weight) test formulation and group 8 animals received NS ( non surfactant ) (8 mg/kg body weight) test formulation. All the control and test samples were administered to animals by intraperitoneal injection. The concentration of drug dopamine HCl in the formulation was of 8 mg/kg body weight. After administration, rats were placed on the rotating rod and the fall off time for 5 minutes was recorded at regular intervals of 30, 60, 90 and 120 minutes. The difference between mean "fall off" time before and after drug treatment was considered for evaluation.

**RESULT**

**Fourier transform infra red spectroscopy (FTIR)**

The spectrum (Figure 1) shows the IR spectra of drug dopamine HCl and the IR spectra of the optimum formulation ST80. Pure drug displays an absorption peak characteristic for the O-H stretching vibration at the range of 3436 $cm^{-1}$ which appeared around 3439$cm^{-1}$ in ST 80 formulation (Table 2). A band with main strong peak at the range of 2925 $cm^{-1}$ is an indicative for the N = H group stretching of amine in the drug which was also present in ST80 formulation at 2926 $cm^{-1}$. The presence of amine ($NH_3$) group in the drug was evidently proved from the ST80 absorption peaks at 1645 $cm^{-1}$. C-H bending of Methyl ($CH_3$) group of the drug showed an absorption peak at the range of 840-843$cm^{-1}$ both in pure drug and ST80 formulation FTIR spectra. These results suggested that there was absence of drug degradation or drug – excipient molecular interaction in its ST80 formulation.

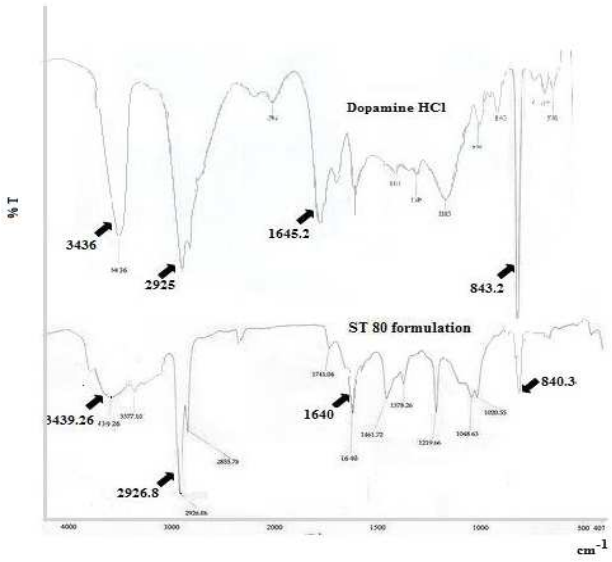


**Figure 1.** Fourier transform infra red spectroscopy for the drug and ST80 formulation

**Table 2. Functional group with their respective absorption peaks for drug dopamine HCl**

| Functional group | Absorption peak | Status * |
|---|---|---|
| 3410 - 3440 | O - H stretching | Present |
| 3015 – 3440 | N=H stretching | Present |
| 1640 – 1645 | $NH_3$ group | Present |
| 840 – 850 | C-H bending | Present |

*Presence of drug's respective absorption peak in FTIR spectrum of drug

### *In vitro* release profile

*In vitro* release studies were performed to predict how a drug delivery system works in ideal situations, thus providing some indications of its release pattern or performance. As the carrier has to initially face biological fluid – blood, PBS pH 7.4 buffer would be ideal to evaluate their release performance to ensure the release behavior of the carrier. *In vitro* release study was carried out for 36 hours which gives a clear indication for the sustained action of the formulation (Figure 2). After 36 hours the cumulative drug release on an average was in the range of 33-38 µg/ml for all the formulation. ST80 formulation followed by S80 showed a greater release than the other formulation after 12$^{th}$ hour. Initially NS showed increased release of the formulation but after 12$^{th}$ hour the release was less compared to the other formulation. The release of drug for S40 was much comparable equal to S80 till 4$^{th}$ hour but later decreased. S20 formulation showed much less cumulative drug release at each hour of evaluation than the other formulation. Till 12$^{th}$ hour, NS showed an enhanced cumulative percentage release of drug in the PBS than ST 80, but after 12$^{th}$ hour, ST 80 showed a potential rise in its release profile on comparing with the NS formuation. After 12 hours, the release study for the surfactant modified liposomes showed an increase pattern of release in the following order, S20<S40<S80<ST80.

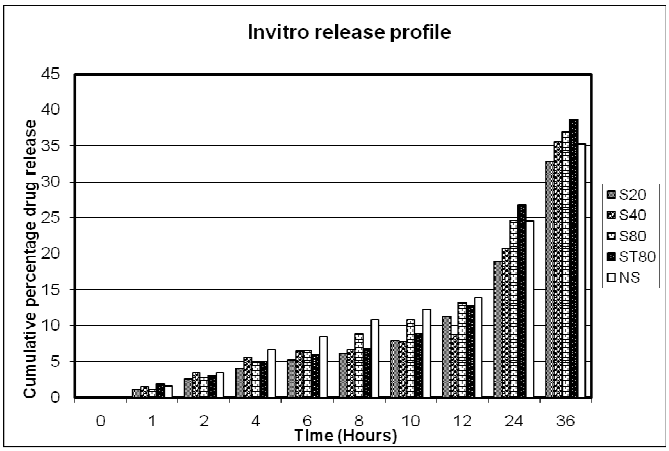


**Figure 2.** *In vitro* release profile of the formulation

### Psychopharmacological Study

The results of psychopharmacological analysis were tabulated in Table 3 and 4 and the percentage of change in locomotor activity from its basal values was represented in Figure 3 and 4. The response of locomotor activity using actophotometer and their respective percentage change of locomotor activity from its basal values were tabulated in Table 3. The "fall off" time from the rotorod after the administration

**Table 3. CNS activity by actophotometer method (response of locomotor activity after the administration of formulation)**

| Group | | #Time interval for evaluation | | | | |
|---|---|---|---|---|---|---|
| | | 0 minute (Basal values) | 30 minutes | 60 minutes | 90 minutes | 120 minutes |
| 1 | Locomotor activity | 108.2±10.8 | 109.3±8.93 | 109.2±8.01 | 106±9.83 | 110.6±8.86 |
| 2 | | 69.3±8.3 | 22.3±6.89 | 23.5±6.22 | 28.6±9.47 | 30.6±8.52 |
| 3 | | 136.9±21.48 | 113.1±20.7 | 123.7±20.57 | 130.4±21.28 | 135.6±20.94 |
| 4 | | 92.1±14.08 | 51.9±17.17 | 50±11.76 | 56.9±12.57 | 65.1±9.01 |
| 5 | | 122.8±5.03 | 64.3±9.35 | 56±9.84 | 62.9±9.53 | 65.7±9.51 |
| 6 | | 98.3±6.85 | 40.8±6.88 | 44.4±7.31 | 49.3±6.83 | 66.9±6.96 |
| 7 | | 112.4±14.26 | 95.1±15.7 | 101.3±14.96 | 106.1±14.19 | 110.8±17.52 |
| 8 | | 91.5±15.64 | 64.3±16.05 | 71.2±18.64 | 78.6±16.68 | 81±15.77 |
| | | $Percentage of change in locomotor activity from its basal values | | | | |
| 1 | % of change in Locomotor activity | - | 0* | 0* | 0* | 0* |
| 2 | | - | 67.82 | 66.08 | 58.7 | 55.84 |
| 3 | | - | 17.38 | 9.64 | 4.74 | 0.94 |
| 4 | | - | 43.97 | 45.71 | 38.21 | 29.31 |
| 5 | | - | 47.63 | 54.39 | 48.77 | 46.49 |
| | | - | 58.49 | 54.83 | 49.84 | 31.94 |
| 7 | | - | 15.39 | 9.87 | 5.6 | 1.42 |
| 8 | | - | 29.7 | 22.1 | 14.09 | 11.47 |

#Values are mean ± SD six animals in each group , comparison were made between group 1 v/s 2, 3, 4, 5, 6, 7 and 8 and P<0.01
$Comparison were made between group 1v/s 2, 3, 4, 5, 6, 7 and 8 and p<0.01
*Not appreciable difference from their respective basal values

**Table 4. CNS activity by rotorod method (response of motor coordination activity after administration of formulation)**

| Group | | #Fall off time (seconds) after the administration of formulation | | | | |
|---|---|---|---|---|---|---|
| | | 0 minutes (BasalValues) | 30 minutes | 60 minutes | 90 minutes | 120 minutes |
| **1** | Fall off time (seconds) | 48.4±25.62 | 43.1±28.14 | 42.1±27.4 | 39.3±25.81 | 47±29.89 |
| **2** | | 85.6±52.12 | 25.3±15.73 | 23.2±14.15 | 28.5±16.54 | 30.3±14.41 |
| **3** | | 164±67.85 | 113.5±56.3 | 139±43.65 | 140±65.81 | 142.3±65.83 |
| **4** | | 125.6±50.11 | 73.3±36.32 | 69.5±31.02 | 85.6±32.78 | 88.3+35.58 |
| **5** | | 126.6±89.62 | 87.1±47.3 | 88.3±55.6 | 93.4±60.59 | 86.6±55.03 |
| **6** | | 158.3±75.62 | 62.6±38.33 | 65.2±33.82 | 69.6±33.12 | 77.1±35.17 |
| **7** | | 129.4±62.64 | 95.5±58.26 | 99.8±58.79 | 105.5±61.46 | 116.3±62.61 |
| **8** | | 115.6±64.85 | 79.8±55.27 | 82±56.76 | 84±56.84 | 84.35±5.41 |
| | | *Percentage of fall off time from its basal values | | | | |
| **1** | % of fall off time (sec) | - | 8.1 | 6.2 | 7.1 | 2.89 |
| **2** | | - | 70.44 | 72.89 | 66.7 | 64.6 |
| **3** | | - | 30.7 | 15.2 | 14.6 | 13.2 |
| **4** | | - | 41.6 | 44.6 | 31.8 | 29.6 |
| **5** | | - | 31.2 | 30.25 | 26.2 | 31.5 |
| **6** | | - | 60.2 | 58.81 | 56.03 | 51.2 |
| **7** | | - | 26.19 | 22.8 | 18.4 | 10.12 |
| **8** | | - | 30.96 | 29 | 27.3 | 27.07 |

#Values are mean ± SD six animals in each group, comparison were made between group 1 v/s 2, 3, 4, 5, 6, 7 and 8 and p<0.01
*Comparison were made between group 1 v/s 2, 3, 4, 5, 6, 7 and 8 and p<0.01

of formulation and their respective percentage of "fall off" time from its basal values were tabulated in Table 4. Statistical analysis for locomotor activity response and "fall off" time response were performed with graphpad instat software (version 3.00, graph pad software, Sandiego, CA) using two way ANOVA followed by chisquare comparison test. Difference with p< 0.01 was considered statistically significant. The ST80 formulation was quite enough in arresting the effect of haloperidol earlier than the other formulation which was evident from the declining response of both percentage of change in locomotor activity (actophotometer) and "fall off" time (rotorod). The control (PBS) showed no

response and animals which has haloperidol only has 55.84% and 64.7% till second hour of fall in response from its basal values for both locomotor activity and fall off time and this indicates negligible experimental error. After first hour of administration of S20 and S40 there was no decline to its basal response but S80 and ST80 showed its potential within half an hour of its administration in combating haloperidol induced parkinsonism by pumping drug loaded liposome in to the brain more efficient and faster than the other formulation. This may be attributed to the flexibility and penetrability of span 80 and tween 80 inclusion in the formulation coupled with transcytosis. But, better response was found for ST80.

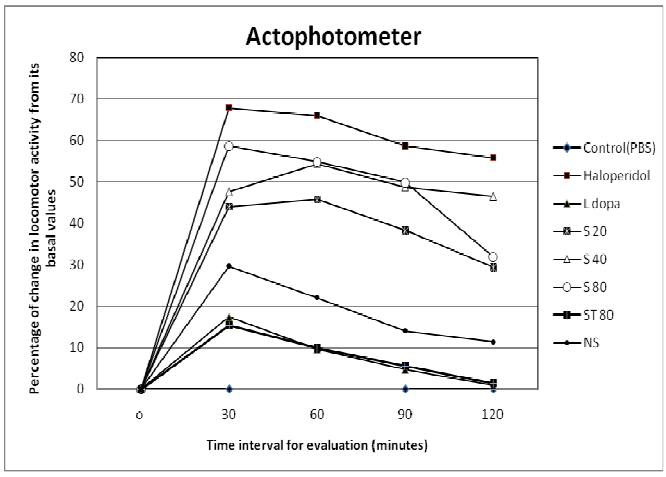


**Figure 3.** CNS activity by actophotometer method

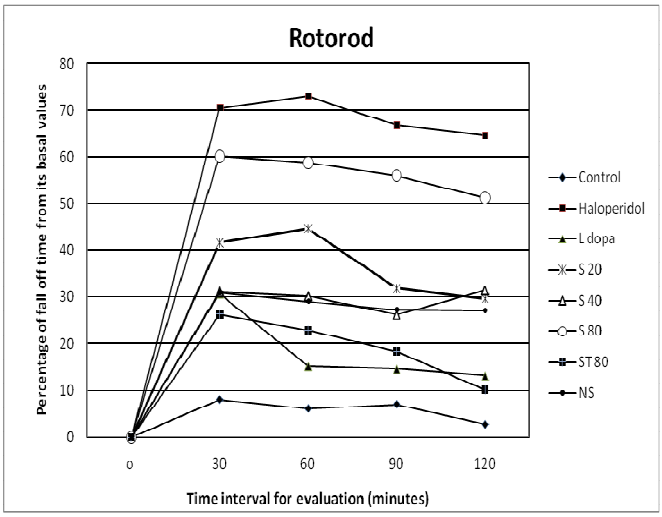


**Figure 4.** CNS activity by rotorod method

## DISCUSSION

Inclusion of surfactants in liposomes produces highly deformable vesicles [18, 19] with transport process reported in few cases depending on the formulation. Surfactants produces flexibility favoring penetration and further increases membrane hydrophilicity and diffusability [20, 21]. Cholesterol in liposomes enhances the rigidity of bilayer membrane above the phase transition temperature of the constituent phospholipids, resulting in increased elastic modulus, which inhibited curving of the bilayer [22, 23]. On inclusion of surfactant, the rigidity of the liposomes was decreased thus proving the increment of fluidity of liposomes. Rigidness of phospholipid carbon could be overcome by this inclusion of surfactants. Tween 80 has very low affinity for lipids and they do not favor vesicle formation (which was evident in fragile or easy collapsible liposome vesicles when prepared using tween 80 only) but with the combination of span 80, MLV was produced which showed comparable stability than tween 80 counterpart.

The crucial feature of the surfactant modified liposomes is that their extreme high flexibility of the membrane to squeeze themselves even through pores much smaller than their own diameter. This is due to the combination of at least two lipophilic / amphiphilic components (phospholipids plus surfactants) with sufficiently different packing characteristics in to a single bilayer [24].

The data obtained from DSC, showed that there was single Tc (transition temperature) for the excipients in presence of drug which was evident for the absence of interaction between the drug and the excipients. This Tc would be indicative for transition of gel phase to liquid phase of lipid mixture [25]. This explicitly shows absence of interference in eliciting drug's pharmacodynamic properties which is in concordant with the findings of Ladbrooke et al. [26] that interaction of the excipients used for the liposomal preparation with that of active drug may alter the physiochemical properties and may also affects the drugs pharmacodynamic properties.

Delivery of neurotransmitter dopamine HCl to brain by the carrier can be affirmed by psychopharmacological studies. Haloperidol induced parkinsonism exhibited extrapyramidal side effects which is sufficient to characterize the features of our surfactant modified or unmodified liposomes. Haloperidol produces dopaminergic neuron receptor block, resulting in drug induced

parkinsonism. Presence of dopamine at considerable amount in postsynaptic dopaminergic receptor could competitively block or remove haloperidol from the receptor [27]. Among the surfactant modified liposomes used, ST80 formulation showed a pronounced effect in delivering dopamine similar to L-dopa drug.

This ST 80 formulation was quite enough in arresting the effect of haloperidol earlier than the other formulation which was evident from the declining response of both percentage of change in locomotor activity (actophotometer) and "fall off" time (rotorod). This may be attributed to the flexibility and penetrability of span 80 and tween 80 inclusion in the formulation coupled with transcytosis. More over P-glycoprotein (P-gp) efflux which is one of the prominent and main barrier for BBB penetration could be blocked by the presence of tween 80 [28] and tween 80 coated or modified nanoparticle are thought to mimic low density lipoproteins (LDL) allowing them to transport across the capillary wall and in to the brain [12]. ST80 formulation because of its high flexibility permits them to squeeze themselves even through pores much smaller than their own diameter without any membrane leakage coupled with defense against P-gp efflux of BBB. In the case of hydrophilic drugs with peripheral side effects, administration of those drugs in these surfactant modified liposomes particularly ST80 could have considerable therapeutic effects making it to be an ideal carrier in ferrying the drug of choice direct in to the brain without any peripheral metabolism.

## CONCLUSION

The present study showed that ST80 formulation which is a deformable lipid vesicle was able enough to ferry a hydrophilic drug dopamine HCl safely to the brain without any peripheral degradation. DSC study showed absence of interaction between drug and excipients. Thus it can be concluded that this surfactant modified liposomal preparation could be an ideal lipophilic and BBB targeted carrier for parkinsonism therapy. This approach of designing brain targeted carrier can also be considered in developing a suitable therapy for any other brain ailments.

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