Development of Solid lipid Nanoparticles of Rivastigmine Tartrate by using full factorial design for the treatment of Alzheimer's disease

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Abstract:
Back ground Study: Solid lipid nanoparticles are attracting importance from drug developers due to their performance, ease of use, flexibility and their potential to produce intellectual property through innovation in drug delivery particularly in the case of modifying drug release systems.
Objective: The objective of present investigation was to develop Rivastigmine Tartrate Solid lipid nanoparticles (SLN) by using full factorial design.
Materials and Methods: The formulations were designed by Design-Expert software. A series of solid lipid nanoparticles (SLN) were prepared by modified solvent emulsification technique using lipid, surfactant, permeation enhancer and other solvents. SLN were evaluated for zeta potential, particle size, polydiispersity index, surface morphology by scanning electron microscopy (SEM) and *in vitro* drug release studies.
Result and Discussion: The particle size of SLN was found in the range of 222±21 to 414±11 nm. Zeta potential of optimized formulation was found in the range of 31.74 ± 3.1 mV indicating stable formulation. SEM photographs indicate discrete spherical structure without any aggregation. Entrapment efficiency was found to be 57.28±1.7%.
Conclusion: The results highlights that the prepared formulation of SLN were able to deliver a constant supply of the active pharmaceutical ingredient (API).
Keywords: Alzheimer's disease, Rivastigmine Tartrate, Solid lipid nanoparticles

INTRODUCTION:

Alois Alzheimer, a German physician is first to identify a brain disorder as Alzheimer's disease (AD) in the year 1906 (1). It is a rare cause of dementia and stands at fourth place in causing death after cancer, heart diseases and stroke. Population with the age of 65 years & over suffering from AD are 10% approximately (2, 3). Population younger than 65 years with AD are around 2,00,000 in New zealand. By 2050, it is estimated that a fresh case likely to develop AD for every 33 s, or almost a million new cases for each year with total estimated occurrence likely to be 13.8 million (4, 5).

Rivastigmine is a cholinergic or parasympathomimetic agent in the curing of mild to moderate AD. It is a cholinesterase inhibitor that inhibits both butyrylcholinesterase and acetylcholinesterase. Destruction of attention and patients memory with AD is related with extensively deficit of acetylcholine. Inhibition of the breakdown of acetylcholine by blocking the enzymes butyrylcholinesterase and acetylcholinesterase with Rivastigmine improves this cholinergic reduction. Thus the administration of Rivastigmine gives established, successful, long-term suggestive treatment in AD patients and Parkinson's disease (PD) patients with dementia (6). Oral bioavailability of Rivastigmine is 36 % and its half life is 1.5 h also patients have tendency to skip the dose. Hence to improve bioavailability, extend duration of action and better patient compliance SLN were prepared.

Nanoparticles are particles between 10-1000 nm in size. Most commonly used nanoparticles are solid lipid nanopaticles (SLN), Polymeric nanoparticles (PNP's), liposomes, magnetic nanoparticles, gold and silver nanoparticles, dendrimers, quantum dots etc. Among these SLN are chosen for this study due to certain advantages over other nanoparticles (7-9).

SLN is an alternative drug delivery systems to colloidal drug delivery systems namely liposomes, oil in water emulsions, microparticles and polymeric nanoparticles. Nanoparticles consist of spherical shape lipid particles in nanometer size range (10-13).

SLN are used for the sustained release and targeted drug delivery for the incorporation of lipophilic drugs and hydrophilic drugs (14-16). SLN are made up of solid lipids, water and emulsifier and/or co-emulsifier. A typical solid lipid is used in such delivery systems melts at temperatures more than body temperature (37 °C).

The present investigation has been mainly focused on preparation of Rivastigmine Tartrate SLN. The optimization of the formulation was done through DoE software.

MATERIALS AND METHODS:
Materials:

Rivastigmine Tartrate was obtained as a gift sample from Jubilant life sciences Ltd, India. Stearic acid and propylene glycol was procured from Loba chemie, Mumbai, India. Poloxamer 188 was purchased from BASF, Germany. All other solvents, reagents and chemicals used were of analytical grade.

EXPERIMENTAL METHOD:
Preparation of Rivastigmine Tartrate SLN (17):

SLN of Rivastigmine Tartrate was prepared by modified solvent emulsification diffusion method. The drug was dissolved in distilled water (internal phase). Small quantity of surfactant (poloxamer 188) and required quantity of lipid (stearic acid) were dissolved in 10 ml of distilled water and heated for 10 minutes and propylene glycol was added to stearic acid solution (external phase). External phase was added to internal phase solution and 10 ml of 70 % aqueous ethanol (co-solvent) was added to above solution and the mixture was homogenized (Polytron PT 1600E, Switzerland) for 15 min at 2000 *g*, and sonicated (Vibra Cell, Model VCX 750, Connecticut, USA) for 10 min. By evaporation technique the organic solvents were removed at 40 °C under normal pressure, and the nanoparticles were separated by using cooling centrifuge (REIL, C-24 BL) for 15 min at 10000 rpm. Supernatant liquid was removed and nanoparticles were washed with distilled water and freeze dried (REMI Ultra low freezer, UDFV-90) using mannitol as cryoprotectant. The Matrix of 3^2 factorial design presented in Table 1.

Table 1: Matrix of 3^2 factorial designs for SLN

Run	Drug (mg)	Factors	
		Lipid (mg)	Surfactant (%)
RS1	12	1.5	0.04
RS2	12	1.5	0.03
RS3	12	0.5	0.02
RS4	12	1.0	0.04
RS5	12	0.5	0.04
RS6	12	0.5	0.03
RS7	12	1.0	0.03
RS8	12	1.5	0.02
RS9	12	1.0	0.02

The corresponding 9 runs of experimental design where the compositions of the lipid and surfactant are indicated. Nine formulations (RS1-RS9) were prepared accordingly and analysed.

Characterization of SLN (17)
Size measurement and polydispersity index (PDI)
The nanoparticles were characterized as such and in diluted form (1:5). Samples were diluted with dust-free ultra-pure water. Zeta potential, conductivity, particle size and PDI were measured at 25°C and at 90° scattering angle.
Scanning electron microscopy (SEM)
The surface morphology of nanoparticles was determined using SEM (Hitachi S3400, Tokyo, Japan). This is to characterize the effect of dilution on the surface morphology of the nanoparticles. The sample of nanoparticles was taken in an aluminum stabu (Plate). The stabu was insulated with a carbon tape and placed in the instrument. Vacuum was applied and SEM photographs were taken.
Determination of entrapment efficiency and drug loading:
A weighed amount (50 mg) of nanoparticles were suspended into 50 ml of ethanol and stirred for 1 h by using magnetic stirrer (Remi, Mumbai, India). It is sonicated for 15 min in order to remove the air bubbles. 1ml of this solution was withdrawn and required dilutions were made using pH 6.8 buffer solution. Absorbance was calculated at wavelength 263 nm.

Fourier Transform Infrared Spectroscopy (FTIR) analysis
Drug and excipients interactions were performed by using FT-IR spectroscopy (Shimadzu FT-IR-8400 spectrophotometer). The samples were prepared by KBr pellet technique, where the samples were dispersed in potassium bromide (KBr) powder, pelletized and analyzed (18).

Differential scanning calorimetry (DSC)
A commonly used thermo-analytical method to generate data on glass transitions and melting endotherms is DSC. All dynamic DSC studies were carried out on DuPont thermal analyzer (2010 DSC module). 3-4 mg of test samples were weighed, captured and sealed hermetically in flatbottomed aluminum pan with lid crimped, followed by positioning these pans on a sample pan holder. Test samples were equilibrated for a minute and then heated in a nitrogen atmosphere over 10-200°C temperature range with 20 °C/min heating rate. Empty aluminum pan served as reference. At the flow rate of 20 ml/min, nitrogen gas was used as purge gas for all studies (19).

***In vitro* drug release Study for SLN:**
The *in vitro* drug release studies of SLN were performed by Franz diffusion type cell. The study was performed at 37 ± 0.5 °C, Receptor compartment of diffusion cell contained 20 ml of 6.8 pH phosphate buffer solution and was continuously stirred by a magnetic stirrer at 100 rpm. Cellophane membrane (molecular weight cut off 10,000-12,000, Hi-Media, India), was employed as release barrier in between receptor and donor compartment which was previously soaked in distilled water. Test samples were withdrawn on definite time intervals from sampling port of the diffusion cell and immediately replaced with an equal volume of fresh buffer. The amount of drug released was quantified using the High performance liquid chromatography (HPLC) method by directly injecting samples to the HPLC system at 263 nm (20-22).

RESULTS AND DISCUSSION:
Determination of Particle size, Zeta potential and Polydispersity index
Rivastigmine Tartrate loaded SLN were evaluated for zeta potential and PDI. The results obtained are graphically represented in figure 1 & 2.
The results indicate indirect relationship between particle size and surfactant concentration influences the particle size of the nanoparticles.
At a concentration of 0.5 % w/v of stearic acid, the particle size of the nanoparticles was found to be 320 nm, 391 nm and 344 nm for RS-2, RS-3 and RS-6 formulations. At 1.0% w/v concentration of stearicacid, the size of nanoparticles was found to be 222 nm, 414 nm and 365 nm

for RS-4, RS-7 and RS-8 formulations. At 1.5% w/v of stearic acid, the particle size was found to be 371 nm, 401 nm and 254 nm for RS-1, RS-5 and RS-9 formulations respectively.

Zeta potential higher than +30 mV indicates the stability of nanoparticulate systems. Zeta potential of stearic acid ans poloxamer 188 nanoparticles depends on the total amino groups on stearicacid. Amino groups interact with acidic groups of sodium alginate for neutralization. The observed zeta potential for the prepared nanoparticles was range of $31.74 \pm 3.1 - 42.23 \pm 2.5$ mV which confirms that the system remained stable without aggregation. Also the net positive zeta potential indicates the presence of free surface amino groups on the nanoparticulate delivery system which will help in initial adhesion to gastric mucosa.

The observed polydispersity index for the prepared nanoparticles was in the range of 0.396 ± 0.12 to 0.781 ± 0.07. since the values are less than 0.8, it indicates narrow size distribution.
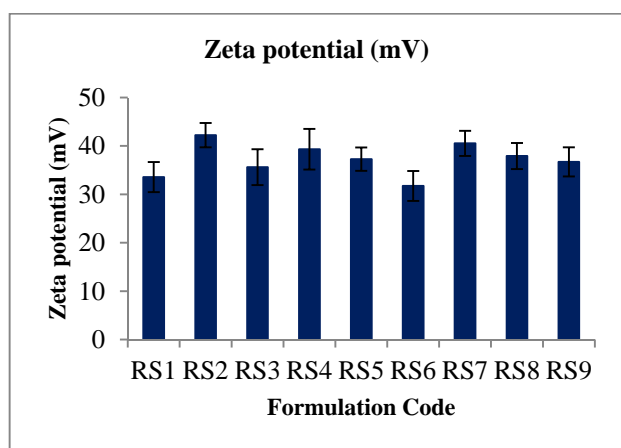

Figure 3: SEM image of SLN

Determination of drug loading and entrapment efficiency

The results of drug loading and entrapment efficiency for all the formulations are represented in figure 4. It is observed from the results that the drug loading is in the range of 17.23 ± 1.4 to 22.23 ± 2.0 % and entrapment efficiency in the range of 42.48 ± 1.8 to 47.28 ± 1.7 %, indicating drug loading is satisfactory.

Figure 1: Zeta potential (mV) of SLN (RS1-RS9)

Figure 4: Drug loading and entrapment efficiency of SLN (RS1-RS9)

***In vitro* drug release studies**

In vitro drug release studies were carried out for all formulations for 24 hours and the results obtained presented in figure 5 and 6.
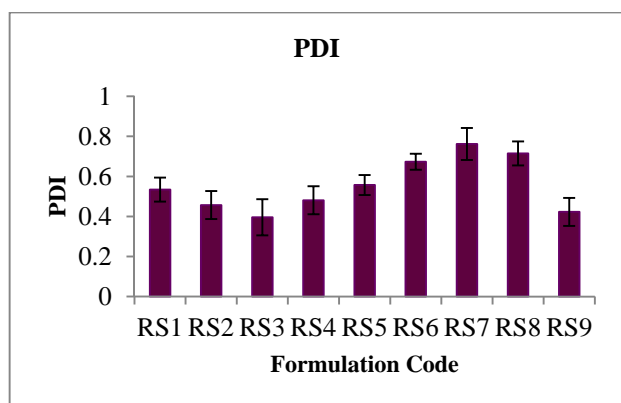

Figure 2: Polydispersity index of SLN (RS1-RS9)

Scanning electron microscopy (SEM)

The scanning electron microscopic photograph of nanoparticles (Figure 3). SEM photographs showed that the nanoparticles were irregular in shape. The observed microporous matrix structures of polyelectrolyte complex can be formed due to electrostatic interactions between anionic groups from lipid and surfactant.
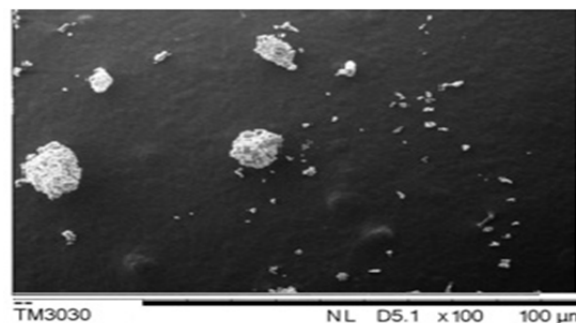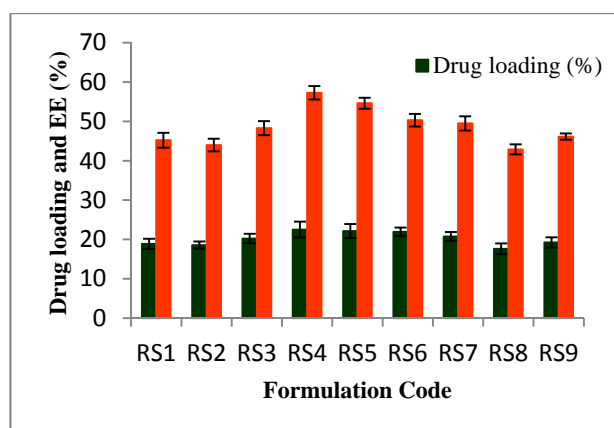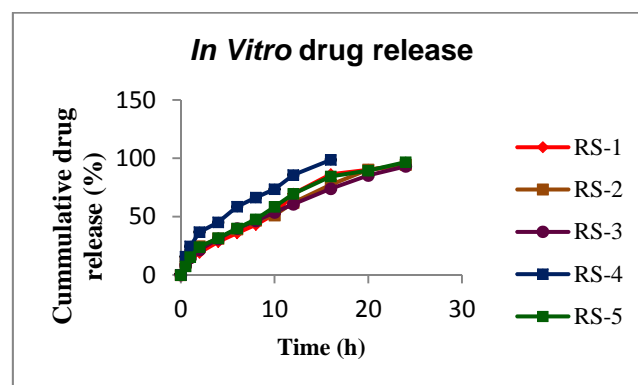

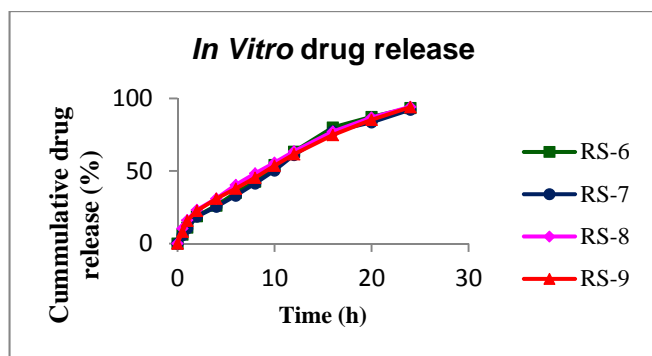**Figure 5:** *In vitro* drug release profile of formulations (RS1-RS5)

Figure 6: *In vitro* drug release profile of formulations (RS6-RS9)

From the results, it was observed that, formulation RS-4 (98.59%) showed maximum release of the drug at the end of 16th h. Formulation RS-1, RS-2, RS-3, RS-5, RS-6, RS-7, RS-8 and RS-9 showed the release upto 94.25%, 94.46%, 93.14%, 96.64%, 93.41%, 92.16%, 94.17%, 93.98%, respectively at the end of 24th h.

The Rivastigmine Tartrate SLN particle size was inversely proportional to the drug release i.e as the size of the nanoparticles decreases; there was increase in the drug release. Lower concentration of lipid and lower concentration of surfactant retarded the drug release whereas higher concentration of surfactant showed faster drug release.

FTIR Spectra:

Figure 7 illustrates the FT-IR spectra of Rivastigmine Tartrate along with its formulation with lipid blend. The FT-IR spectrum of Rivastigmine Tartrate showed C-H symmetric stretching of the -ane group at 1165.04 cm^{-1}, C-N stretching of amine group at 1265.35 cm^{-1}, C=O (carbonyl group) symmetric stretching at the 1597.11 cm^{-1}, -C-N (amide group) stretching at 2908.75 cm^{-1}. All the characteristic peaks of Rivastigmine Tartrate along with minor shifts were shown by spectra of the mixture of Rivastigmine Tartrate and excipients. The FT-IR spectrum of mixture of Rivastigmine Tartrate and excipients showed C-N stretching, C=O stretching, and –C-N stretching at 2918.40 cm^{-1}, respectively. These data evidently indicate that the characteristic absorption peaks of Rivastigmine Tartrate were seen in physical mixture of drug and excipients, and it indicates that drug Rivastigmine Tartrate and excipients had no interaction between them.
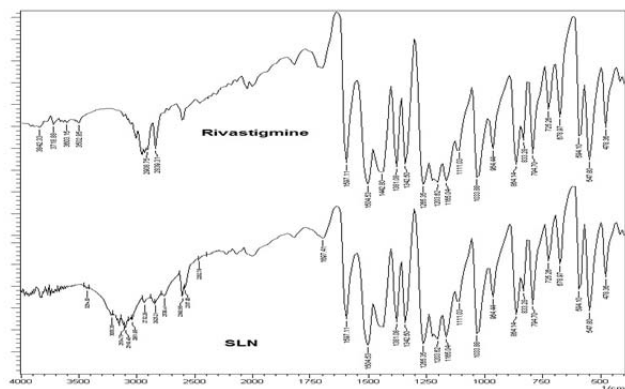

Figure 7: FTIR Spectra of Pure Rivastigmine Tartrate and optimized formulation

DSC Studies:

DSC studies were carried out for Rivastigmine Tartrate and optimized formulation (RS-10). The thermograms obtained are presented in figure 8. The data obtained from the dynamic DSC scans are given in table 2. The temperature T_O, T_m and T_c are respectively the onset of melt, the melting point and the completion of the compound.
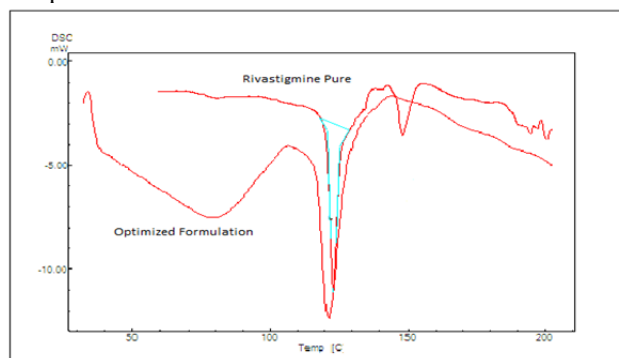

Figure 8: DSC thermograms of Rivastigmine Tartrate and optimized formulation

Table 2: DSC thermogram data for Rivastigmine Tartrate and optimized formulation

Drug and Formulation	To(°C)	Tm(°C)	Tc (°C)	Melting range (°C)
Rivastigmine Tartrate	121.4	127.3	130.8	9.4
Optimized formulation	118.7	124.4	131.2	12.5

Where, To – Onset of melt, Tm - Melting point and Tc – Completion of melt

By conducting a group of preliminary trials with relative ratio of the selected two components i.e. Stearic acid and Poloxamer 188 on the previous related experiences, the upper and the lower limits of each variable were defined (Table 3). To evaluate all the possible combination of excipients in the initial formulation system, a full factorial DOE of studies is required (Table 4).

Table 3: Variables in 3^2 factorial designs for Rivastigmine Tartrate SLN

Independent variables	Levels		
	Low (%)	Medium (%)	High (%)
A: Stearic acid	0.5	1.0	1.5
B: Poloxamer 188	0.02	0.03	0.04
Dependent variables S1: Particle size S2: Entrapment efficiency S3: Cummulative drug release			

The result (Table 6) depicts that variables chosen have strong influence on the selected responses, as particle size (nm), entrapment efficiency (%) and percentage cumulative drug release values were in the range of 222 - 414 nm, 42.89 - 57.28% and 73.87 - 98.59 % respectively.

Table 4: Observed response in 3^2 factorial design for Rivastigmine Tartrate SLN

Run	Factors		Responses		
	Lipid (%)	Surfactant (%)	Particle size (nm)	Entrapment efficiency (%)	Cummulative drug release (%) (16th hr)
RS1	1.5	0.04	321±14	45.21±1.9	86.30±0.54
RS2	1.5	0.03	371±12	43.98±1.6	77.33±0.72
RS3	0.5	0.02	396±18	48.27±1.8	73.87±0.81
RS4	1.0	0.04	222±21	57.28±1.7	98.59±0.56
RS5	0.5	0.04	254±16	54.62±1.4	84.52±0.58
RS6	0.5	0.03	344±23	50.30±1.6	79.98±0.74
RS7	1.0	0.03	365±17	49.48±1.8	77.38±0.46
RS8	1.5	0.02	414±11	42.89±1.3	77.15±0.74
RS9	1.0	0.02	401±27	46.14±0.8	74.81±0.81

* mean ± SD, n=3

Full factorial design Results:

Design of experiments (DOE) is a scientific approach applied to understand the process in a larger way and to resolve how the input influences the response. In the present work, 3^2 factorial design was applied to study the effect of variable on the selected responses. Every excipient is included to suit the needs of product use and processibility.

The application of factorial design yielded the following regression equations.

A. **Particle size** = + 512.77778 + 0.3733 * Lipid - 690.00000 * Surfactant

B. **Entrapment efficiency (%)** = + 45.81722 - 0.070367 * Lipid + 33.01667 * Surfactant

C. **C Drug release** = + 58.51000 + 8.03333E – 003 * Lipid + 72.63333 * Surfactant

Where negative values indicate a negative effect of a specific variable on the response factor and positive value indicates positive effect of a specific variable. The polynomial regression results were expressed using Contour graphs, predicted & actual graphs and 3-D graphs (Figure 9 - 11)

i. Particle size (nm)

The regression equation depcits, the effect of stearic acid and poloxamer-188 on particle size. It clearly shows that at higher concentration of stearic acid, the particle size increases and as the concentration of surfactant increases, the particle size decreases. It shows that a synergistic effect exists if the regression equation for a response parameter shows positive value while antagonistic effect in case it is negative.
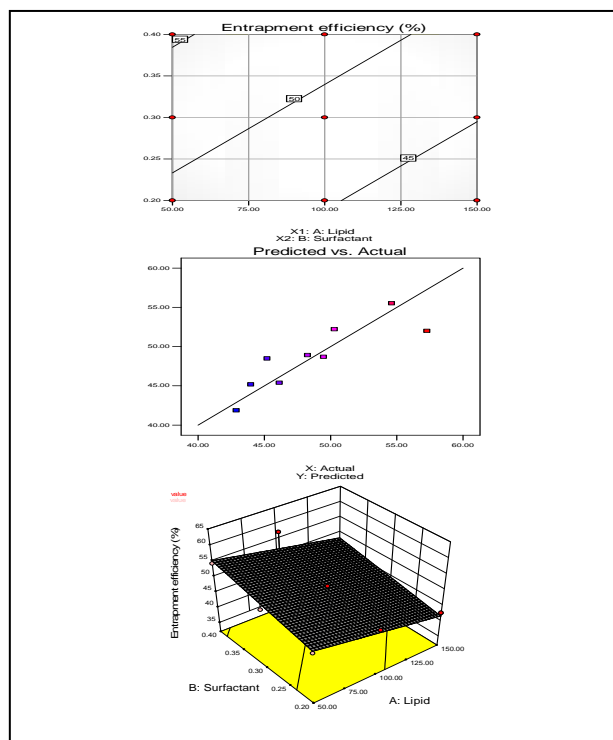
ii. Entrapment efficiency (%)

The regression equation depcits, the effect of stearic acid and poloxamer-188 on entrapment efficiency, and the obtained result indicate a positive effect on entrapment efficiency. As the concentration of stearic acid and poloxamer-188 increases and the entrapment efficiency is increases.

iii. Cummulative drug release

Results of regression analysis shows that, particle size was inversely proportional to the drug release i.e as the size of the nanoparticle decreases, an increase in the drug release was observed. Higher concentration of lipid and higher concentration of surfactant retarded the drug release whereas higher concentration of surfactant showed faster drug release.
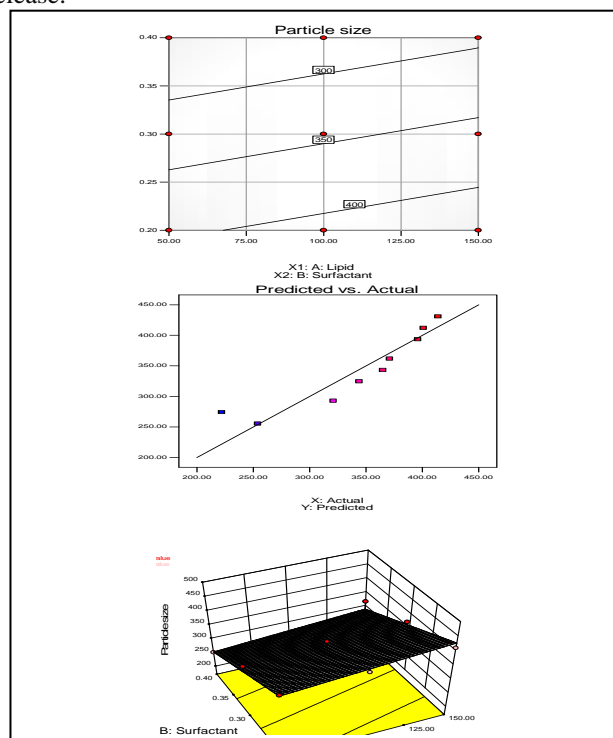
Figure 9: Contour plot, Predicted V/S actual plot and Three-dimensional response surface plot depicting the impact of Lipid and Surfactant on particle size (nm) respectively

Figure 10: Contour plot , Predicted V/S actual plot and Three-dimensional response surface plot depicting the impact of Lipid and Surfactant on Entrapment Efficiency (%) respectively
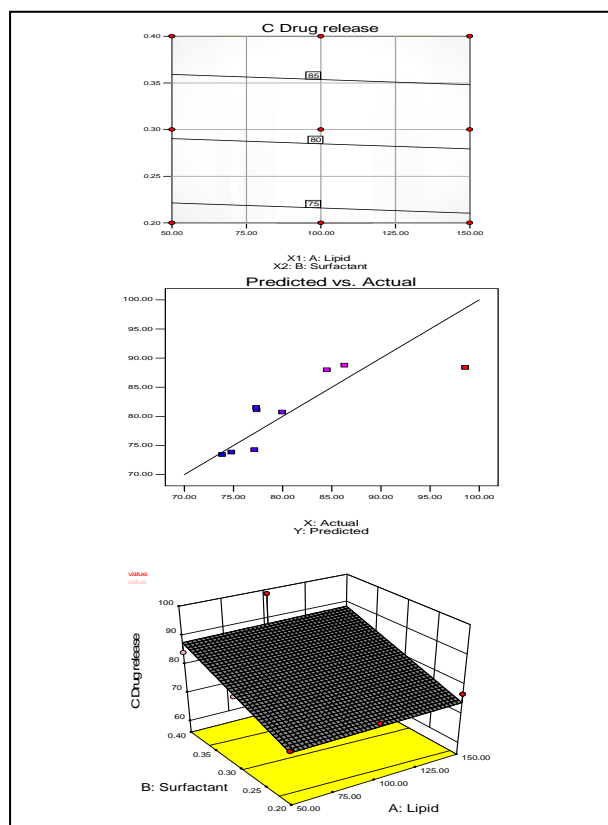
Figure 11: Contour plot , Predicted V/S actual plot and Three-dimensional response surface plot depicting the impact of Lipid and Surfactant on Drug release (%) respectively

CONCLUSION:

In the present work, Rivastigmine Tartrate SLN was successfully prepared by modified solvent emulsification diffusion technique. The various physicochemical properties and the *in vitro* release behavior were greatly affected and can be controlled by optimizing the compositional variables represented in the concentration of surfactant and lipid as well as the type of lipid used. The sustained release behavior of Rivastigmine Tartrate SLN with favorable physicochemical characteristics can form a foundation for further clinical studies using these nanoparticles for the transdermal delivery of Rivastigmine Tartrate.

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