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RESEARCH ARTICLE

# Formulation and Characterization of Rivastigmine Loaded Solid Lipid Nanoparticles

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## ABSTRACT

In the present study, we explored the potential of Rivastigmine loaded solid lipid nanoparticle (SLN), as a new formulation in improving the bioavailability of Antialzheimer drug Rivastigmine which otherwise reported with poor bioavailability. The "Micrioemulsion based method" was adopted for preparation of SLN. A $3^2$ full factorial experiment was designed to study the effect of independent variables such as lipid, surfactant and co-surfactant composition. The formulations were lyophilized to get free flowing powder. The mean particle size of SLN measured to be 137 – 1300 nm with PDI value of 0.590 – 1.279, and zeta potential value of -3.27 to -27.31 mV was observed which indicates SLN formulations found to more stable. The entrapment efficiency was estimated to be 92.82 – 99.80%. SEM study shows SLN in spherical as well as irregular in shape. DSC and FTIR results also confirmed the molecular encapsulation of drug in the lipid matrix. The in-vitro release study shows that all formulations followed Higuchi's Classical Diffusion Model which implies that developed formulations have a potential to deliver the drug in controlled release manner. These finding explore the potential of proposed SLN of rivastigmine formulation as an alternative drug delivery system in improving bioavailability of Rivastigmine.

## KEYWORDS

Alzheimer Disease, Rivastigmine, Stearic acid, Preformulation, SLNs, Characterization, Particle size, SEM, Zeta Potential, Kinetic studies

## INTRODUCTION

Alzheimer disease (AD) is most common prevalent neurodegenerative disorder. Today, it affects nearly 30 million people in the whole world. With each passing year about 4 million people in the world develop dementia. As the average population increases, the number of AD patients is expected to rise exponentially and about 110 million of patients are projected for 2050.

There are some common features suggesting that in AD brain could be an acceleration of processes occurring in aged brain. Adult neurogenesis occurring in the dentate gyrus (DG), a process that decreases in aged mammals and that could be related with loss of memory, an important feature in AD.

A loss in declarative memory has been found in patients with AD. In these patients, neurodegenerative at the hippocampal region

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takes place at the first steps of the disease. In normal ageing there is a mild cognitive impairment, but this impairment could be accelerated in AD.[1]

Alzheimer's disease (AD) applied to a state of presenile dementia, extra-neuronal protein aggregations (plaques), and intraneuronal protein aggregations (tangles). Although it was recognized at the time that brains of persons with senile dementia could also manifest plaques and tangles, in the elderly this was not felt to represent an actual disease state.[2]

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder that begins with cognitive and memory impairments, accompanied with behavioural disturbances such as aggression, depression, hallucination, delusion, anger and agitation and eventually progresses to dementia, physical impairment and death.[3]

**Rivastigmine Tartrate**

Chemically Rivastigmine tartrate is N-Ethyl-N-methylcarbamic acid 3-[(1S)-1-(dimethylamino) ethyl]phenyl ester (2R,3R)-2,3-dihydroxybutanedioate.[4]

Rivastigmine tartrate is a white to off-white powder.[5] It is very soluble in water, soluble in ethanol and acetonitrile, slightly soluble in n-octanol and very slightly soluble in ethyl acetate. It has molecular formula $C_{14}H_{22}N_2O_{22}.C_4H_6O_6$ having molecular weight 400.43 g/mol.[6]

Rivastigmine tartrate is a reversible (or pseudoirreversible because it separates too slowly from AChE) nonselective cholinesterase inhibitor which inhibits both AChE and BuChE in the central nervous system (CNS). It binds both esteratic and ionic sites of AChE just like a natural substrate, and it inhibits the metabolism of Ach. It is 4-6 times more effective on the G1 (monomeric) form of the enzyme, which is present at higher concentrations in the brain of AD patients. There is no affinity of rivastigmine tartrate for muscarinic, alpha- or beta-adrenergic, or dopamine receptors or opoid binding sites.[7]

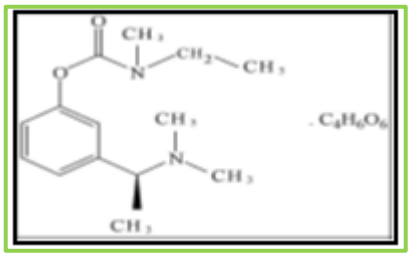

Figure 1: Structure of Rivastigmine Tartrate

**Aim and objective of present research work**

Presently rivastigmine tartrate is available in the form of tablet, capsule containing 1.5mg, 3mg, 4.5mg, 6mg and the common side effects associated with oral administration (gastrointestinal) like vomiting, diarrhoea, increased acid secretion in stomach and reduced heart rates. Oral administration shows significant first-pass effect. Its half-life is about 1.5 hrs.

Rivastigmine tartrate is also available in the form of transdermal patch containing 4.5mg, 9.5mg and the common side effects associated with transdermal route are allergic reactions such as hives, difficulty in breathing, swelling (face, lips, tongue or throat), pale skin, necessitating drug discontinuation.

This inherent drawback of oral and transdermal rivastigmine tartrate administration warrants an alternative drug delivery system for rivastigmine tartrate. Hence in the present work an attempt is being made to provide an alternative colloidal drug delivery system for rivastigmine tartrate in the form of solid lipid nanoparticles which will have the following advantages

- ✓ Sites specificity and controlled drug release.
- ✓ Protection of drug against chemical degradation.
- ✓ High drug pay load.
- ✓ Ease of manufacturing.

In the present work an attempt has been made to develop SLN of rivastigmine tartrate by micro-emulsification method and evaluate it for the following;

1. Preformulation studies on drug and polymer and to establish their compatibility in formulation using FT – IR.
2. To prepare solid lipid nanoparticles of rivastigmine tartrate.
3. Evaluation of the formulation for
   - Physical characterization of the solid lipid nanoparticles which includes
     - Particle size Analysis
     - Determination of Particle shape and Surface morphology
   - Percentage yield
   - Drug entrapment efficiency
   - *In-vitro* drug release study
   - Release kinetics

## MATERIAL & METHODS

Rivastigmine tartrate was purchased from Swapnroop Drugs & Pharmaceuticals, Aurangabad, Maharashtra, India. Stearic acid was purchased from Loba Chemicals, Mumbai. Poloxamer 188 (BASF, Germany) Supplied by RFCL limited, Mumbai. All other chemicals and solvents used were of analytical grade.

### Instrument Used

UV-Visible double beam spectrophotometer Shimadzu UV1800 with 1cm matched quartz cells. Electronic Balance. IR Spectrophotometer, Magnetic Stirrer, High speed propeller, Particle size analyser, Scanning Electron Microscope, Differential Scanning Colorimetry, Zeta potential

### Preformulation Studies[8]

Preformulation testing is the first step in the rational development of dosage forms of the drug.

The goals of preformulation studies are

- To establish its compatibility with different excipients.
- To establishment the necessary physicochemical characteristic of a new drug substance.
- To determine its kinetic release rate profiles.

Hence, preformulation studies carried out with pure sample of drug include physical tests (description, melting point & solubility) and compatibility studies (drug with excipients).

### Preparation of Calibration Curve

100 mg of rivastigmine tartrate was accurately weighed and dissolved in 100 ml water and methanol mixture (9:1) in volumetric flask, the resultant solution gives the concentration of 1mg/ml i.e.1000 µg/ml (stock solution-I). From this 10 ml solution was taken and then diluted up to 100 ml with the same solvent in a volumetric flask and then the concentration of this stock will be 100µg/ml (stock solution-II). From this stock solution-II10,20,30, 40, 50, 60, 70, 80, 90 and 100ml solutions were pippetted and volume was made to 100 ml using water to get concentrations of 10,20,30, 40, 50, 60, 70, 80, 90 and 100µg/ml respectively. The absorbance of these solutions was measured at 221 nm.

### Compatibility Studies

A proper design and formulation of a dosage form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Before producing the actual formulation, compatibility of rivastigmine tartrate with different polymers and other excipients were tested using the Infrared Spectroscopy (IR) technique and Differential Scanning Colorimetry (DSC).

### FTIR Spectroscopy[9]

IR spectra of rivastigmine tartrate alone and along with excipients, this final complex were determined by Fourier Transform Infrared spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then obtained mixtures were taken in a diffuse reflectance sampler and spectra were recorded by scanning

in the wavelength region of 500 to 5000 $cm^{-1}$ in a FTIR Spectrophotometer.

**Differential Scanning Calorimetry (DSC) [10]**

DSC was performed in order to assess the thermo-tropic properties and thermal behaviour of the drug and the complex compacts prepared. A sample of 2-3mg was accurately weight was subjected to DSC run over the temperature range 40-350°C.

**Preparation of Solid Lipid Nanoparticles[13]**

The SLNs were prepared by Microemulsion based method. A 32 full factorial design was utilized in the present study for the development SLNs. Rivastigmine loaded SLNs were prepared from a warm o/w microemulsion containing Stearic acid as internal phase, poloxamer188 as surfactant and sodium taurocholate as co-surfactant. Microemulsion prepared by melting lipid (stearic acid) at 50ºC with measured quantity of drug, followed by sonication. To this poloxamer 188 was added and stirred for 2 min. Aqueous phase containing co-surfactant (sodium taurocholate) heated at 50ºC and added to melted lipid phase with mechanical stirring for 10 to 15 min, results in o/w microemulsion. This microemulsion was then added carefully dropwise into ice cold water present in a beaker with continuous stirring. Factors such as rate of addition, distance of needle from the surface of the beaker, rate of stirring were standardized to reduce particle size. In order to obtain optimum microemulsion, the needle was placed 4cm from the surface of the water and mixture stirred at 3000 rpm. The SLN dispersion was further stirred for 3hr after the complete addition of micro-emulsion. After completion of stirring, the SLN dispersion was subjected to ultra-sonication for a period of 10 min.

The nine batches (3x3) of SLN were prepared by varying the lipid concentration, surfactant concentration and co-surfactant concentration, using 32 factorial designs in three batches as shown in Table 1.

Table 1: Formulation Table of Rivastigmine Tartrate Loaded Solid Lipid Nanoparticles

| Batch | Formⁿ | Drug (mg) | Stearic acid (mg) | Poloxamer 188 (mg) | Sodium taurocholate (mg) |
|---|---|---|---|---|---|
| Batch 1 | F1 | 50 | 250 | 150 | 30 |
| | F2 | 50 | 500 | 150 | 45 |
| | F3 | 50 | 750 | 150 | 60 |
| Batch 2 | F4 | 50 | 250 | 225 | 45 |
| | F5 | 50 | 500 | 225 | 68 |
| | F6 | 50 | 750 | 225 | 90 |
| Batch 3 | F7 | 50 | 250 | 300 | 60 |
| | F8 | 50 | 500 | 300 | 90 |
| | F9 | 50 | 750 | 300 | 120 |

**Evaluation and Characterization of the Prepared Solid Lipid Nanoparticles[12-17]**

**Percentage Yield**

The practical percentage yield was calculated from the weight of solid lipid nanoparticles recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\text{Percentage Yield} = \frac{\text{Practical Mass (SLNs)}}{\text{Theoretical Mass}} \text{ X } 100$$

**Particle Size and Surface Morphology Analysis**

Particle size analysis was done by using particle

size analyser. Surface morphology was done by using Scanning Electron Microscopy (SEM).

### Determination of Percentage Entrapment Efficiency

Entrapment efficiency of rivastigmine tartrate loaded solid lipid nanoparticles was estimated by centrifugation method. The prepared solid lipid nanoparticles were placed in centrifugation tube and centrifuged at 15000 rpm for 30 min. The supernatant (1ml) was withdrawn and diluted with water + methanol (9:1). The unentrapped rivastigmine tartrate was determined by UV spectrophotometer at 221 nm and calculated by following formula.

$$\%E.E = \frac{\text{Total amount of drug} - \text{Free dissolvsed drug}}{\text{Total amount of drug}} \text{ X } 100$$

### *In vitro* Drug Release Studies

### Drug Release

*In vitro* dissolution studies were carried out in 900 ml of phosphate buffer 7.4 as a medium using USP apparatus type II (basket type). The rotation speed was 50 rpm and a temperature of 37±0.5˚C was maintained. The samples were analyzed by UV double beam spectrophotometer at λ 221 nm. Cumulative percentages of drug dissolved from solid lipid nanoparticles were calculated and graphs were plotted.

### Release Kinetics

The data of *in-vitro* study was fitted in to three different kinetic models namely zero order kinetic model, first order kinetic model, Higuchi's classical kinetic model. The mechanism of drug release is defined statistically in terms of co-relation co-efficient the highest values of co-relation co-efficient signify the particular release mechanism.

### Zeta Potential

Zeta potential is an important and useful tool to indicate particle surface charge. Zeta potential was carried for all formulations of Rivastigmine SLNs.

## RESULTS AND DISCUSSION

### Preformulation Studies

The drug sample of rivastigmine tartrate was found to bewhite to off white powder having melting point 123 - 125°C and very soluble in water, soluble in ethanol and acetonitrile.

### Compatibility Study

### Physical Compatibility Study

Table 2: Result of drug excipients physical compatibility study after 15 days at 37ºC±2°C / 75%RH± 5 % RH

| Sr. No. | Drug + Excipients | Initial Observation | After 15days at 37ºC±2°C / 75%RH ±5 %RH |
|---|---|---|---|
| **1** | Drug: Rivastigmine tartrate | White to off-white powder | Compatible |
| **2** | Stearic acid | A white to off white pellets | Compatible |
| **3** | Poloxamer 188 | White to off white powder or solid prill | Compatible |
| **4** | Drug + Stearic acid | A white powder | Compatible |
| **5** | Drug +Stearic acid + poloxamer 188 | A white to off white Creamy powder | Compatible |

### FTIR Compatibility Study

IR spectra of drug and polymer were obtained, which are depicted in Figure 2. All the characteristic peaks of rivastigmine tartrate were present in spectra at respective wavelengths (Table 3). Thus, indicating compatibility between drug and polymers. It shows that there was no significant change in the chemical integrity of the drug.

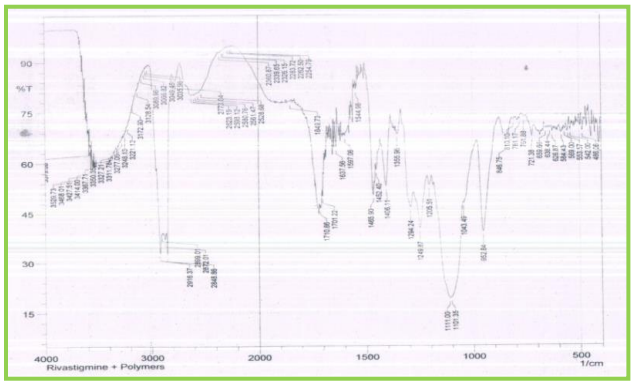


Figure 2: IR Spectra of Mixture of Rivastigmine Tartrate + Stearic Acid + Poloxamer 188

Table 3: Peaks ($Cm^{-1}$) And Functional Groups Present – Rivastigmine Tartrate + Steric Acid + Poloxamer 188

| Sr. No. | Peaks $cm^{-1}$ | Functional group |
|---|---|---|
| **1** | 1597.06 | C = C (Stre) |
| **2** | 3172.90 | C – H (Stre) |
| **3** | 1849.73 | C = O (Stre) |
| **4** | 1294.24 | C – O (Stre) |
| **5** | 1544.98 | Alkyl group |

## DSC Compatibility Study

The results of DSC analysis showed that the melting temperature for rivastigmine tartrate was found to be 113.30°C. The details of thermograms are shown in Figure 3. There was no significant changes observed.

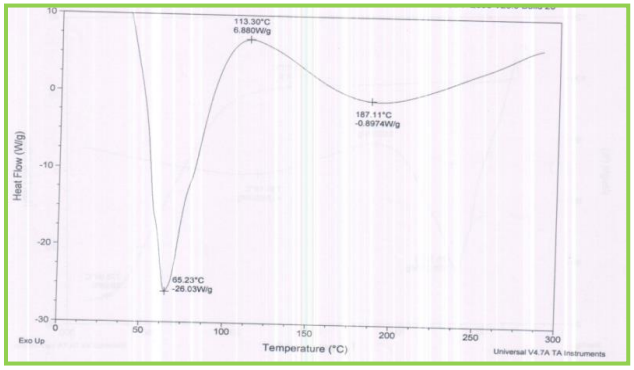


Figure 3: DSC Thermogram of Rivastigmine Tartrate + Stearic Acid + Poloxamer188

## Determination of $\lambda_{max}$

The $\lambda_{max}$ of rivastigmine tartrate was determined in water and methanol mixture (9:1) which was scanned between 200-400nm in the UV spectrometer. It was found to be 221nm.

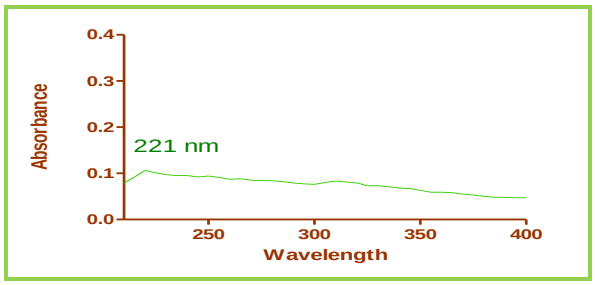


Figure 4: λmax of rivatigmine tartrate

## Standard Calibration Curve for Rivastigmine Tartrate

Calibration curve for rivastigmine tartrate was constructed using water + methanol (9:1) as solvent at 221nm.The concentration selected was 10 – 90 µg/ml (Table 4, Figure 5).

Table 4: Calibration data for rivastigmine tartrate

| Concentration (µg/ml) | Absorbance (nm) |
|---|---|
| 10 | 0.102 |
| 20 | 0.214 |
| 30 | 0.303 |
| 40 | 0.415 |
| 50 | 0.512 |
| 60 | 0.601 |
| 70 | 0.727 |
| 80 | 0.819 |
| 90 | 0.909 |

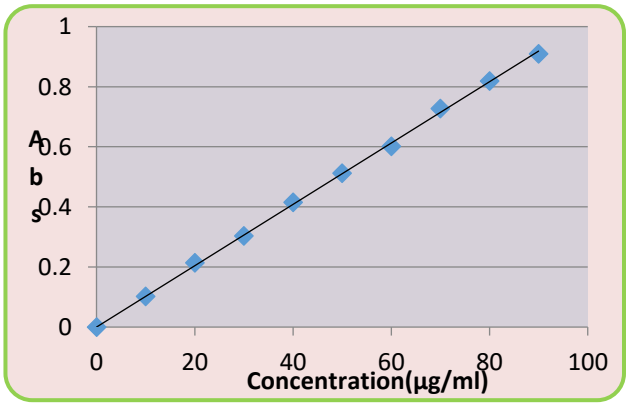


Figure 5: Standard Calibration Curve of Rivastigmine Tartrate

**A straight line was obtained at $R^2$=0.999. Equation of straight line was found to be y= 0.010x**

### Percentage Yield

The percentage yields of all nine formulations were calculated and were affected by concentration of polymer and the ratio of the mixture of polymers. The increase in polymer concentration leads to increase in percentage yield. The percentage yields of all formulations are shown in Table 5.

Table 5: Percentage Yield of Solid Lipid Nanoparticles of Rivastigmine Tartrate

| Formulation code | Percentage yield (%) |
|---|---|
| F1 | 55.66 |
| F2 | 66.36 |
| F3 | 72.8 |
| F4 | 54 |
| F5 | 64.72 |
| F6 | 70.37 |
| F7 | 50.33 |
| F8 | 60.36 |
| F9 | 67.37 |

### Particle Size Analysis

The mean particle size ranged from nm137 - 1300nm .The mean size was influenced by the concentration of lipid, surfactant and co-surfactant used in the formulations.

This may be due to the less availability of amphiphiles during emulsion formation and may be partly due to more partitioning of surfactant into oil phase as the concentrations of aqueous phase was increased. The particle size of SLNs decreases with increase in the concentration of poloxamer188. An increase in the concentration of sodium taurocholate leads to decrease the particle size of SLNs. Sodium taurocholate has the ability to decrease the size of the particles. Mean particle size of all formulations are given in the Table 6 and its graphical representation were shown in Figure 6 to Figure 14. The average mean particle size of all formulations were shown in Figure 15.

Table 6: Mean Particle Size and Polydispersity Index of Formulations

| Formulation Code | Mean Particle Size (nm) | PDI |
|---|---|---|
| F1 | 1300 | 0.837 |
| F2 | 194.7 | 0.980 |
| F3 | 137.5 | 0.590 |
| F4 | 531.0 | 0.938 |
| F5 | 242.8 | 1.119 |
| F6 | 212.2 | 1.279 |
| F7 | 609.0 | 1.250 |
| F8 | 191.0 | 0.890 |
| F9 | 175.8 | 0.970 |

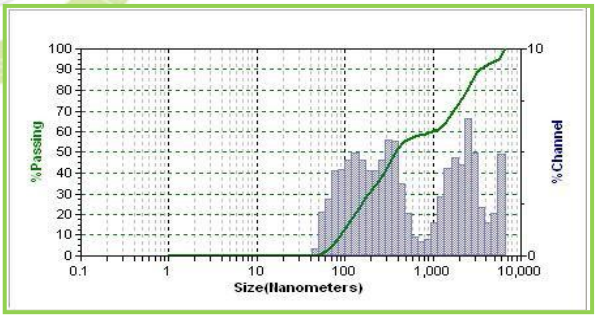


Figure 6

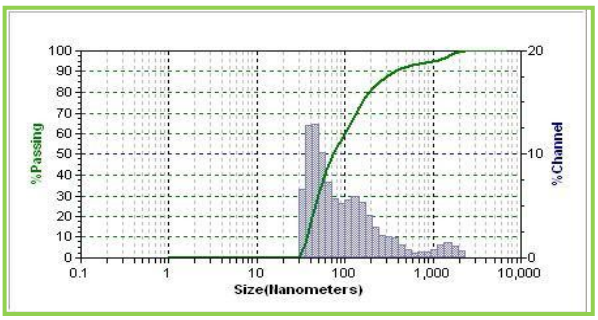


Figure 7

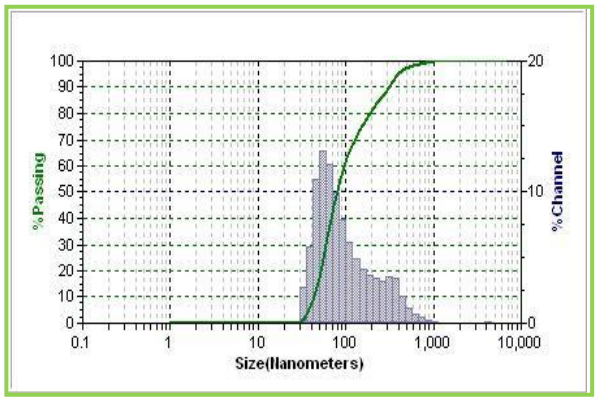


Figure 8

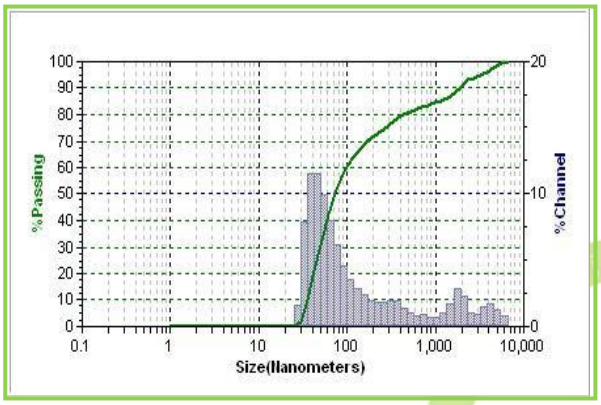


Figure 9

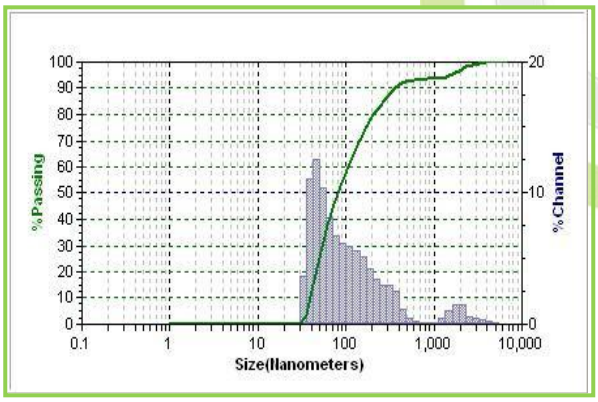


Figure 10

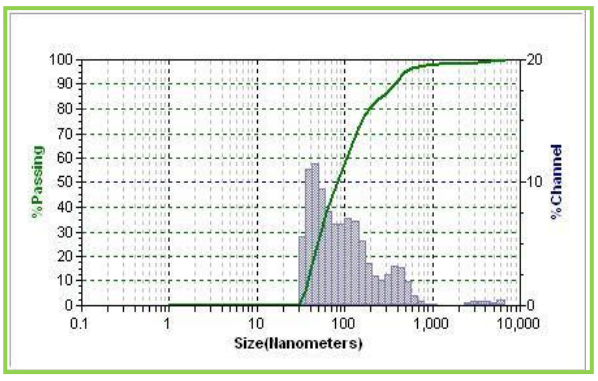


Figure 11

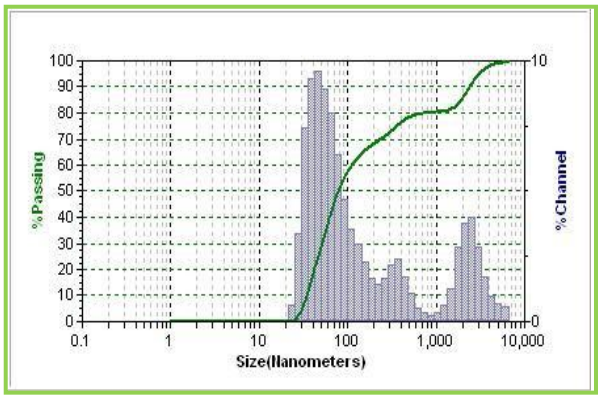


Figure 12

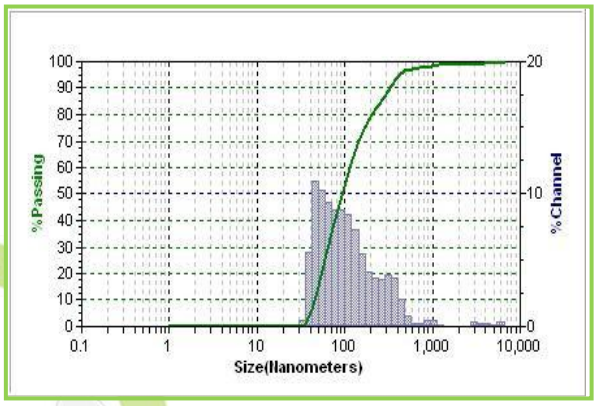


Figure 13

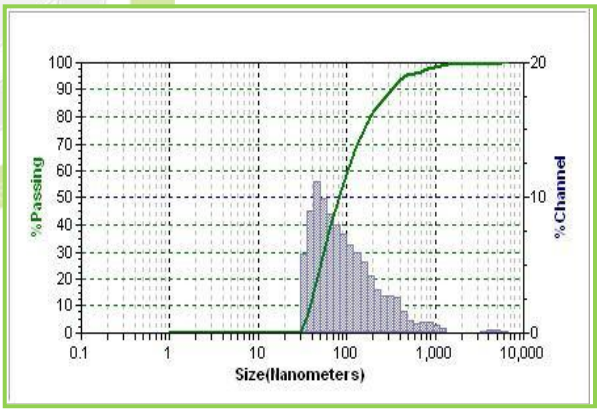


Figure 14

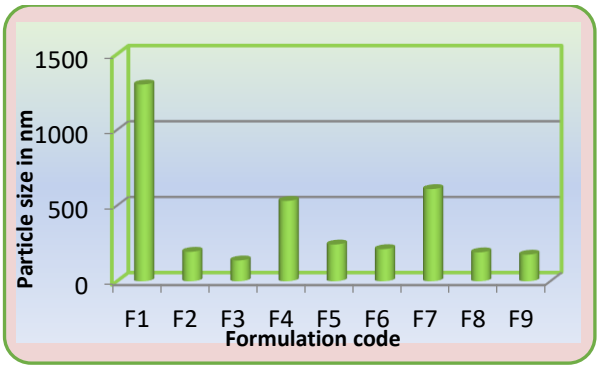


Figure 15: Average Particle Size

### Shape and surface Morphology

Solid lipid nanoparticles of rivastigmine tartrate were found to be spherical and irregular and their surface was smooth and devoid of cracks giving them good appearance. The SEM data obtained on the drug-loaded solid lipid nanoparticles of F9 shown in Figure 16.

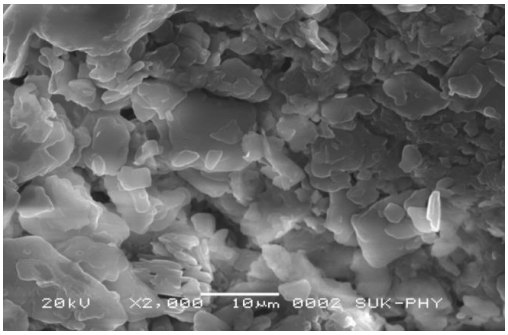


Figure 16: SEM Image of Rivastigmine Tartrate Loaded SLNs

### Drug Entrapment Efficiency

The drug entrapment efficiency of a rivastigmine tartrate in sold lipid nanoparticles ranged from 93.26% to 99.80% (Table 7). It was observed that, when lipid concentration increased the entrapment efficiency was found to increase.

Table 7: Drug Entrapment Efficiency of Different SLN Formulations

| Formulation code | Entrapment efficiency (%) |
|---|---|
| F1 | 93.26 |
| F2 | 95.26 |
| F3 | 97.82 |
| F4 | 92.82 |
| F5 | 96.04 |
| F6 | 97.90 |
| F7 | 93.35 |
| F8 | 96.58 |
| F9 | 99.80 |

### Comparison of Formulations

Table 8 and Figure 17 shows comparison of % yield, % entrapment efficiency and particle size.

Table 8: Comparison of Percentage Yield, Drug Entrapment Efficiency, Particle Size of Solid Lipid Nanoparticles of Rivastigmine Tartrate

| Formulation code | % Yield | % Drug entrapment efficiency | Particle size (nm) |
|---|---|---|---|
| F1 | 55.66 | 93.26 | 1300 |
| F2 | 66.36 | 95.62 | 194.7 |
| F3 | 72.87 | 97.82 | 137.5 |
| F4 | 54 | 92.98 | 531.0 |
| F5 | 64.72 | 96.04 | 242.8 |
| F6 | 70.37 | 97.90 | 212.2 |
| F7 | 50.33 | 93.35 | 609.0 |
| F8 | 60.36 | 96.58 | 191.0 |
| F9 | 67.37 | 99.80 | 175.8 |

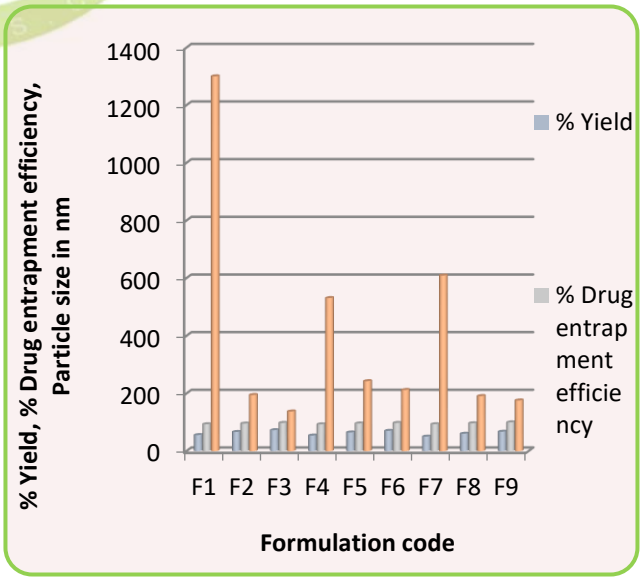


Figure 17: Comparison of % Yield, Particle Size and %Drug Entrapment Efficiency

### *In-vitro* Drug Release

### Release Kinetic Data for Solid Lipid Nanoparticle Formulations

The data obtained from *in vitro* drug release studies were fitted to zero-order, first-order and Higuchi's equations and is represented in Figure 18, 19 and 20 respectively. After performing statistical analysis for release study data the coefficient of correlation was found to favour Higuchi's classical diffusion model.

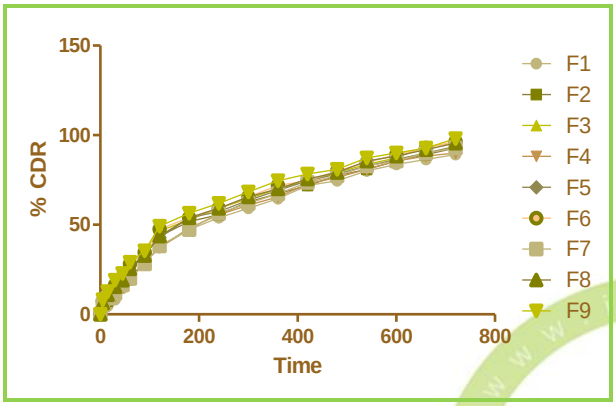


Figure 18: Zero order kinetic

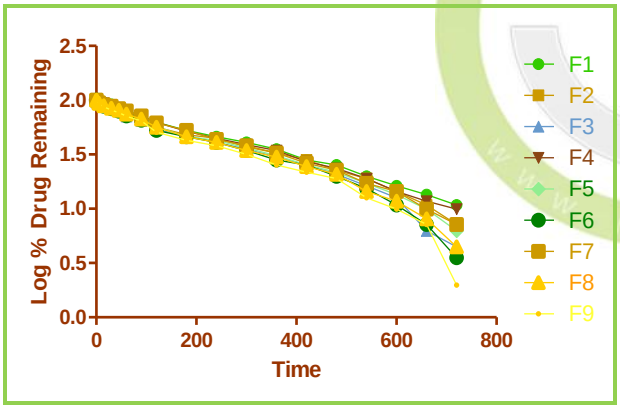


Figure 19: First order kinetic

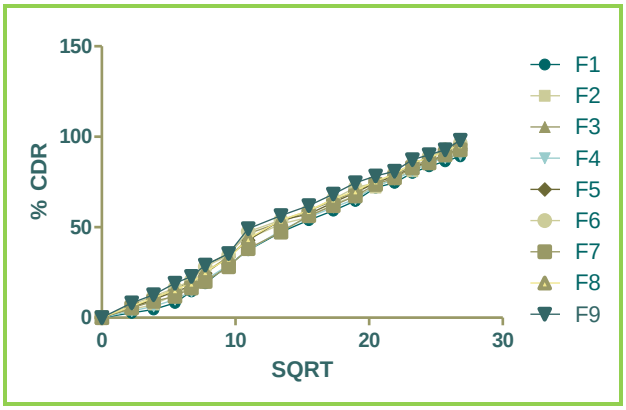


Figure 20: Higuchi's diffusion model

The values for regression coefficient shown in Table 9 for different kinetic models. From the results it is seen that the drug release mechanism from the formulation was found to follow Higuchi's classical diffusion model. The rate of drug release is related to the rate of diffusion. The dissolution process is purely defined that the release rate is depends on the diffusion of drug from the lipid matrix, present in the developed formulation.

### Zeta Potential

The zeta potential values obtained for the rivastigmine tartrate SLNs whichare given in Table 10 shows that the formulated rivastigmine tartrate SLNs are stable. F6 formulation was more stable than the other formulations.

Table 10: Zeta Potential of Rivastigmine Loaded Solid Lipid Nanoparticles

| Formulation code | Zeta Potential(mV) |
|---|---|
| F1 | -3.27 |
| F2 | -4.37 |
| F3 | -7.48 |
| F4 | -13.36 |
| F5 | -19.27 |
| F6 | -27.31 |
| F7 | -24.43 |
| F8 | -22.41 |
| F9 | -21.32 |

## CONCLUSION

In the present work, solid lipid nanoparticles of rivastigmine tartrate were formulated to deliver rivastigmine in a controlled manner. A satisfactory attempt was made to develop solid lipid nanoparticles of rivastigmine tartrate and evaluated for *in vitro* characterization studies.

From the study following conclusions could be drawn.

- Rivastigmine loaded SLNs were prepared successfully, and the process parameters were optimized using $3^2$ factorial design.
- The preformulation studies involving description, solubility, melting point of the drug were found to be comparable with the standard. Based on all the above preformulation studies, the drug rivastigmine tartrate was suitable for preparation of drug loaded solid lipid nanoparticles.
- Drug-polymer compatibility studies by FT-IR and DSC gave confirmation about their purity and showed no interaction between the drug and selected polymers.
- Practical and percentage yield increased as the concentration of lipid added increased.
- Particle size studies revealed that mean size of the prepared SLNs was in the size range of 137nm -1300nm and particles were spherical & irregular in shape.
- By varying the concentration of lipid, it was found that increase in lipid, surfactant (poloxamer188) and co-surfactant (sodium taurocholate) concentration in formulation leads to decrease in particle size, and increase in percentage entrapment efficiency and controlled release rate.
- By performing *in vitro* drug release study it was observed that the drug release from the formulations increases as the particle size of the formulation decreases.
- Rivastigmine tartrate release from all formulations followed Higuchi's classical diffusion model kinetics.
- Zeta Potential shown that the F1 formulation was more stable than others.

This outcome from release profiling strongly recommends that developed rivastigmine tartrate loaded solid lipid nanoparticles can be useful delivery carrier to deliver drug in controlled release manner.

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